ASSESSMENT OF ANTIMALARIAL ACTIVITY AND IMPACT OF AQUEOUS METHANOL LEAF EXTRACT OF *SENNA OCCIDENTALIS* ON MARKERS OF KIDNEY AND LIVER FUNCTION

BY

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DECLARATION

I hereby declare that is work is the product of my own research efforts undertaken under the supervision of Professor A J. Alhassan and has not been presented and will not be presented elsewhere for the award of degree or certificate. All sources have been duly acknowledged.

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CERTIFICATION

This is to certify that the research work for this project and subsequent preparation of this thesis by **SHEHU MUAZU AHMAD** with registration number **SPS/15/PBC/00030** was carried out under my supervision.

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Professor A .J. Alhassan
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APPROVAL

This research work has been examined and approved as part of meeting the requirements for the award of Master of Science degree in Biochemistry of Bayero University, Kano.

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DEDICATION

This research work is dedicated to my mother Hajiya Amina and my lovely father Alh. ShehuMuazu Abbas, my entire family and finally my wife Ramlatu Muhammad, May Allah (S.W. A) Reward them with jannatul Firdausi.

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Abstract

The high rate of resistance to antimalarial drug suggest the importance to discover new compounds with potential antimalarial activities.This research work is aimed at evaluating the antimalarial activity and assessing impact of aqueous methanol extract of *Senna occidentals* leaves (ESOL) on liver and kidney markers as well as characterisation of potential bioactive compounds.The *in vitro* antimalarial activity of the aqueous methanolic, ethyl acetate, chloroform and hexane fraction of ESOL was carried against *Plasmodium falcifarum* using method decribed by mukhtar 2006, The antimalarial activity of the extracts was determined by calculation of the percentage elimination of the ESOL fractions after three day of incubation against *Plasmodium falcifarum*.Analysis for the detection of possible bioactive compound (s) in the ESOL was conducted using Gas chromatography Mass spectrometry (GCMS).For the assessment of impact of aqueous ESOL on markers of liver and kidney functions,a total of 20 wistar rats were grouped into four groups; Groups I, II, II and IV administered with 0 (as normal control), 100, 200 and 300mg/kg body weight of the Aqueous-ESOL respectively.Results showed that ECOLhave antimalarial properties that were dose dependent.Furthermore, there was a significant increase ($p<0.05$) in mean percentage elimination of all the extract when compared with placebo (normalsaline). All the fractions of ESOL shows an activity less than the conventional drug, ArtemisininCombination Therapies(*ACT),*with the chloroform extract showing highest antiplasmodial activity of 94.64% at 5000 µg/ml. Chloroform fraction of ECOL was found to contain containUrs-12-en-3-ol,acetate(3 beta), (alpha amyrin),Lup-20(29)-en-3-ol, acetate and 12-Oleanen-3-yl acetate, (beta amyrin acetate) as possible bioactive compounds. In addition,treatment with aqueous-methanol ESOL caused asignificant decrease ($p<0.05$) in the mean serum activities of Aspartate Aminotransferase(AST),Alanine Aminotransferases Assay (ALT) ,Alkaline Phosphatase (ALP) and Albumin (ALB) **,**and increase in Direct bilirubin (DB) and Total bilirubin (TB) compared to control.This suggests that the extract do not have adverse effect on the kidney. Similarlythere wassignificant increase ($p<0.05$) in mean serum creatinine, urea,sodium and potassium while a decrease ($p<0.05$) was observed in chloride and bicarbonate of groups administered with 200 and 300mg/kg body weight compared to the control. This study suggests that the aqueous methanol ESOL may not be toxic to wistar rats in the doses administered.The study also revealed that the ESOL have potential anti-plasmodial activity.

CHAPTER ONE

GENERAL INTRODUCTION

1.1 BACKGROUND OF STUDY

It is a common practice in Nigeria and other parts of the world to use plant in the form of crude extracts, decoction, infusion or tincture to treat common infection and chronic conditions. According to WHO, over 70% of the world populations rely on medicinal plants for primary health care (WHO, 2008) and there are reports from various researchers on natural substances of plant origin which are biologically active, with desirable antimicrobial and antioxidant properties (Hamid *et al.,* 2010). Despite tremendous progress in orthodox medicines, infectious diseases caused by bacteria, fungi, viruses and parasites are still a major threat to public health. Their impact is particularly large in developing countries due to relative unavailability of medicines and the emergence of widespread drug resistance (Zampini *et al.,* 2009). During the last two decades, the development of drug resistance as well as the appearance of undesirable side effects of certain antibiotics has led to the search for new antimicrobial agents mainly among plant extracts with the goal to discover new chemical structures which overcome the above disadvantages (Okemo *et al.,* 2005).

Traditional medicine (indigenous medicine or folk medicine) describes medical knowledge systems developed over centuries within various societies before the era of modern medicine. The World Health Organization (WHO, 2008) defines traditional medicine as the health practices, approaches, knowledge and belief incorporating plant, animal and mineral based medicines, spiritual therapies, manual techniques and exercises, applied singularly or in combination to treat, diagnose and/or prevent illness or maintain well-being (WHO, 2008).

Medicinal plants are plants that possess therapeutic properties or exert beneficial pharmacological effect on animals. Although there are no apparent morphological characteristics

`in the medicinal plants, growing them is medicinally important. It is now been established that the plants, which naturally synthesizes and accumulate some secondary metabolites e.g alkaloids, glycosides, tannins, volatile oils and certain minerals and vitamins possess some medicinal properties (Shimbe and Tor-Anyiin 2014).

1.1.1 Malaria parasite

Malaria is estimated to kill more than 1 million people annually, the majority of whom are young children. Together with pneumonia, diarrhoea, measles and malnutrition, malaria is responsible for over 70% of deaths in young children especially in developing countries (Unicef, 2000).Malaria is mostly a disease of hot climate. In 2017, 91 countries and territories had ongoing malaria transmission. According to the latest WHO estimates, released in December 2017, there were 216 million cases of malaria in 2017 and 445000 deaths (WHO, 2017). Most malaria cases and deaths occur in sub-Saharan Africa. Ninety per cent of malaria cases in the world occur in Africa south of the Sahara.

In Nigeria, malaria is endemic throughout the country, accounting for up to 60% outpatient visits to health facilities, 30% childhood mortality and 11% maternal deaths (WHO, 2014). Malaria is a vector borne disease, caused by protozoan parasites of the genus Plasmodium. It is transmitted from the blood of an infected person and passed to a healthy human by a female anopheles mosquito bites (WHO, 2014).

The Anopheles mosquito, which transmits the malaria parasite from one human being to another, thrives in warm, humid climates where pools of water provides perfect breeding grounds. It proliferates in conditions where awareness is low and where health care systems are weak*(WHO, 2013*; Fujioka and Aikawa, 2002*).* Five species of *Plasmodium* can infect and be spread by human species. The species *P. knowlesi* rarely causes disease in humans (WHO, 2014). It is

typically transmitted through the bite of an infected anopheles mosquito which carry the *Plasmodium* parasite.

The parasite is released into the bloodstream. Once the parasites are inside the body, they travel to the liver, where they mature. After several days, the matured parasites enter the bloodstream and begin to infect red blood cells and within 48 to 72 hours, the parasites inside the red blood cells multiply, causing the infected cells to burst open. The parasites continue to infect red blood cells, resulting in symptoms that occur in cycles that last two to three days at a time.

1.1.2 Drug resistance

Malaria chemoprophylaxis especially in chloroquine resistant *P. falciparium* areas has become a real problem. The attempts to secure protection under these circumstance with the utilization of amodiaquine, the combination of sulfadoxine/pyrimethamine (Fansidar), sulfalene/pyrimethamine (Metakelfin), or pyrimethamine/dapsone (meloprim), halfan, halofantrin with or without chloroquine had to be abandoned or to be used with caution in view of the severe complications following the weekly administration of these drugs (Onori and Majori,1998). Anti-malarial drug resistance, particularly *P. falciparium* resistance has been a major setback in the fight against malaria and its attendant complications (Wongsrichanalai *et al.,* 2002). Plants have been the basic sources of sophisticated traditional medicine systems for thousands of years and were instrumental to early pharmaceutical drug discovery and industry (Elujoba *et al.,* 2015). An appreciable level of studies has been done on African traditional medicinal plants. However, in Nigeria, particularly in the northern part, studies on the extraction and perhaps testing of the effects of these herbal extracts on malarial parasites have been

minimal. In other words not much have been scientifically proved of the antimalarial activity of indigenous *Senna occidentalis* in our community.

The fact that traditional medicinal plants used for the treatment of malaria and typhoid fever provided the lead compounds for synthetic antimalarial in use currently, gives hope that plants particularly those used in the unorthodox treatment of the disease may provide new lead compounds for development of new and novel antimalarial drugs. It is for this reasons that *senna occidentalis* popularly known by "*rai dore"* which is usually used by our local traditional people in mostly northern of Nigeria for the treatment of malaria and other diseases was selected to study its anti plasmodial activities, characterise the most active components and as well study its toxic effect on the kidney and the liver parameters.

1.1.3 *Senna occidentalis*

Senna occidentalis is widely distributed and commonly used plant. *Sennaoccidentalis*, commonly called 'Dora rai' in Hausa, 'Akidiogbara' in Igbo, 'Abo rere' in Yoruba and 'Coffee senna' in English has been reported to contain many phytochemicals including alkaloids, anthocyanosides, phenolics, proteins, phlobatannins, steroids, tannins, flavonoids, anthroquinone, saponins, terpenes, resins, balsams, amino acids, carbohydrates, sugars and cardiac glycosides (Alhassan *et al.,* 2017). *Senna occidentalis* have been reported to have many pharmacological effects including antimicrobial, anthelmintic, insecticidal, antioxidant, antianxiety, antidepressant, antimutogenic, antidiabetic, wound healing, hepatoprotective, antiinflammatory, analgesic, antimalarial ,antipyretic and other effect . The plant is widely used by the local people of Hausa-Fulani tribe in northern Nigeria ` for the prevention and treatment of various diseases liver and kidney diseases inclusive (Alhassan *et al.,* 2017).

It is a tropical plant that grows on wastelands in villages and towns and on roadsides. The seeds are the primary material of interest though the leaf and roots are also used. The seeds are roasted and used as a coffee substitute. The plant's tissues contain a host of phytoactive chemicals that may support its numerous applications in folk medicine (Francis, 2002). The whole plant is useful as a purgative and as a tonic. The seeds and leaves are used as cure for cutaneous diseases (Yadava, 2011). The roasted seeds are used to manage hypertension in Ghana. It is used for fever, menstrual problems, tuberculosis, diuretic anemia, liver complaints, and as a tonic for general weakness and illness (Usha and Katsuri2007). Leaves of *S.occidentalis* are externally applied for wound healing, itching, bone fracture, ringworm, skin diseases and throat infection. An infusion of the bark is used in folklore medicine for diabetes (Reeta *et al.,* 2013).

Considering the documented therapeutic properties of *Senna occidentalis*, the present work will be undertaken to evaluate the in vitro antimalarial of aqueous-methanol leaves extracts of *Senna occidentalis*. Although the toxic effects of the seeds of *Senna occidentalis*is wellcharacterized, the toxicity of other parts of the plant is not well determined. Therefore, this research will also investigate further the possible toxic effect of the leaves of *Senna occidentalis* in order to ensure its safety intake and as well characterize the most active compound present in it.

1.2 Statement of the Research Problem

Malaria remains one of the most devastating infectious diseases with approximately 207 million infections and more than 600,000 deaths each year - primarily children under the age of 5 in sub-Saharan Africa.*Plasmodium falciparum,* the deadliest form of the malaria parasite, is responsible for the vast majority of the mortality and morbidity associated with malaria infection. Malaria is highly endemic in Nigeria where it accounts for 60% outpatient visits to health facilities, 30%

childhood death and 11% of maternal death(4500 die yearly)(NDHS, 2016). These days, the term alternative medicine is becoming very common. Plants are a rich source of many natural products most of which have been extensively used for human welfare, and treatment of various diseases. The world has relied on plants for the best malaria drugs: chloroquine from Cinchona tree; and Artemisinin from Chinese salad plant, Artemisia annua. Artemisinin Combination Therapies, or ACTs, are currently the frontline treatments against *P. falciparum* malaria. Although these treatments are working well in many parts of the world, there is serious concern that malaria parasites are once again developing widespread resistance to this vital treatment. As the threat of antimalarial drug resistance grows, there is increasing pressure to sustain the efficacy of existing treatments, as well as developing alternative treatments.

1.3 Justification

People prefer to use medicinal plants over allopathic medicine for various reasons, relatively low cost, effectiveness, perceived safety and minimal side effects (Fransworth and Fabricant, 2001). Currently used antimalarial drugs such as artemisinin and quinine based drug's parent mole`cules were identified and isolated from medicinal plants. The above mentioned drugs were discovered from traditional setting in treating malaria associated symptoms. In general the cost of modern drugs has recently increased due to the higher cost of the materials. It is a widely accepted fact that the cost of orthodox drugs are increasing faster than medicinal plants in Kano State. Because of the above problems, it becomes necessary to explore. Alternative methods of treating malaria in the state. This present an opportunity to explore ethnomedicinal plants for new antimalarials.

1.4 Aim of the study

This research work is aimed at evaluating the antimalarial activities of the extracts of *Senna occidentalis*leaves and its impact on the markers of liver and kidney function in rats.

1.5 objectives of the study

The objectives are to:

1. Evaluate *in vitro* antimalarial activities of aqueous-methanol extract of*Senna occidentalis* leaves.
2. Fractionate and evaluate the activity of the partitioned solvent fractions against *Plasmoduim falciparum.*
3. Identify the most active fraction and characterize the possible active component.
4. Assess impact of aqueous-methanol extract of*Senna occidentalis* leaves on markers of liver and kidney functions in rats.

CHAPTER TWO

LITERATURE REVIEW

2.1 HISTORY OF MALARIA

The symptoms of malaria were described in ancient Chinese medical writings. In 2700 BC, several characteristic symptoms of what would later be named malaria were described in the Nei Ching, the Canon of Medicine. Nei Ching was edited by Emperor Huang Ti. Malaria became widely recognized in Greece by the 4th century BC, and it was responsible for the decline of many of the city-state populations. Hippocrates noted the principal symptoms. By the age of Pericles, there were extensive references to malaria in the literature and depopulation of rural areas was recorded (Reiter, 1999). Until the second half of the 20th century, malaria was endemic and widespread in many temperate regions, with major epidemics as far north as the Arctic Circle. From 1564 to the 1730s the coldest period of the Little Ice Age malaria was an important cause of illness and death in several parts of England (Reiter, 1999).

In the Susruta, a Sanskrit medical treatise, the symptoms of malarial fever were described and attributed to the bites of certain insects. A number of Roman writers attributed malarial diseases to the swamps. Charles Louis Alphonse Laveran, a French army surgeon stationed in Constantine, Algeria, was the first to notice parasites in the blood of a patient suffering from malaria. In 1882, he went to Rome with the special aim of seeking, in the blood of patients who had become infected with malaria in the Roman Campagna, the parasites he had found in the blood of patients in Algeria. His researches, conducted at the San Spirito Hospital, confirmed his opinion that the blood parasites that he had described were in fact the cause of malaria. His first communications on the malaria parasites were received with much scepticism, but gradually confirmative researches were published by scientists in every country and, in 1889, the Academy

of Sciences awarded him the Bréant Prize for his discovery. For his discovery, Laveran was awarded the Nobel Prize in 1907. In 1894 Ronald Ross, was determined to make an experimental investigation in India of the hypothesis of Laveran and Manson that mosquitoes are connected with the propagation of the disease. After two and a half years' failure, Ross succeeded in demonstrating the life-cycle of the parasites of malaria in mosquitoes, thus establishing the hypothesis of Laveran and Manson (Tans and Sung, 2008). The Italian investigators Giovanni Batista Grassi and Raimondo Filetti first introduced the names *Plasmodium* vivax and *P. malariae* for two of the malaria parasites that affect humans in 1890. Laveran had believed that there was only one species, *Oscillaria malariae.* An American, William H. Welch, reviewed the subject and, in 1897, he named the malignant tertian malaria parasite *P. falciparum.* There were many arguments against the use of this name; however, the use was so extensive in the literature that a change back to the name given by Laveran was no longer thought possible (Lindemann, 1999). In 1922, John William Watson Stephens described the fourth human malaria parasite, *P. ovale. P. knowlesi* was first described by Robert Knowles and Biraj Mohan Das Gupta in 1931 in a long-tailed macaque. The first documented human infection with *P. knowlesi* was in 1965 (Julia *et al.,* 2006).

2.1.1 Symptoms of Malaria

The symptoms of malaria typically develop within ten days to four weeks following the infection. In some people, symptoms may not develop for several months (Darla, 2005). Some malarial parasites can enter the body but will be dormant for long periods of time. Common symptoms of malaria include: shaking chills that can range from moderate to severe, high fever, profuse sweating, headache, nausea, vomiting, diarrhea, anemia, muscle pain, convulsions, coma, bloody stools (Julia*et al.,* 2006). Malaria can cause a number of life-threatening complications also. The following may occur: swelling of the blood vessels of the brain, or cerebral malaria, an accumulation of fluid in` the lungs that causes breathing problems, or pulmonary edema, organ failure of the kidneys, liver, or spleen, anemia due to the destruction of red blood cells, and low blood sugar (Darla, 2005). Among these, is the development of respiratory distress, which occurs in up to 25% of adults and 40% of children with severe *P. falciparum*malaria. Although rare in young children with severe malaria, acute respiratory distress syndrome occurs in 5–25% of adults and up to 29% of pregnant women (Taylor *et al.,* 2012). Malaria in pregnant women is an important cause of stillbirths, infant mortality, abortion and low birth weight, particularly in *P. falciparum* infection, but also with *P. vivax* (Hartman *et al.*, 2010).

2.1.2 Life Cycle of Malaria Parasite

The disease remains one of the major killers of humans worldwide, threatening the lives of more than one third of the world's population (Smith and McKenzie, 2004). Humans contact malaria from the bite of a *Plasmodial*- infected female *Anopheline* mosquito (Killeen *et al.,* 2000). As the mosquito inserts its proboscis into a human to take its blood meal, it injects the plasmodial sporozoite at the same time through its saliva (Killeen *et al.,* 2006). The sporozoite begins the

asexual cycle by the pre-erythrocytic development of merozoites in the parenchymal cells of the liver (Tsuji *et al.,* 1994). The merozoites can repeat the pre-erythrocytic cycle in the liver cells, or they can enter the erytrocytic cycle. Once the merozoites penetrate the erythrocytes, the parasite undergoes several morphological changes. First, a ring form develops, which enlarges to become a mature amoeboid trophozoite filling most of the parasitized red blood cell (Philip, 2011). Next, asexual multiplication takes place by the splitting of nuclear material and cytoplasm of the amoeboid-appearing parasite to form more merozoites. Depending on the species, this multiple fission (schizogony) results in 6 to 36 new merozoites per parasitized erythrocyte. As the erythrocyte ruptures, the merozoites are freed into the blood plasma to infect many other erythrocytes. During the erythrocytic cycle, some merozoites differentiate as male and female gametocytes (Shortt *et al.,* 1951; Zhu and Hollingdale, 1991). Each merozoite invades a red `blood cell, and for two days multiplies into more merozoites. The red blood cell full of merozoites ruptures to release more merozoites. It is this stage of the life cycle that causes disease and, too often, death. Some merozoites change into the form called gametocytes, which do not cause disease but remain in the blood until they are cleared by drugs or the immune system, or taken up by the bite of a mosquito.

For the sexual cycle to evolve, the gametocytes of both sexes must be ingested in the blood meal of another female Anopheles mosquito. In the gut of the mosquito, the male gametocyte forms spermatozoa, and the female forms an ovum. Fertilization of the ovum takes place, and the resting zygote changes shape, becomes motile, and invades the gut wall. Next, in the tissues of the gut wall, sporogony of the parasite takes place. That is, there is multiple fission of parasitic content, and numerous sporozoites are formed. The sporozoites migrate through the tissues of the mosquito to the salivary glands where they wait to be injected into another unsuspecting human

host when the mosquito takes its next blood meal (Saul *et al.,* 1990; WHO, 1999; Celement, 2000). The asexual cycle begins again, and malaria is established in a new host.

2.1.3 Global Disease Burden

According to the latest estimates from WHO, there were 214 million new cases of malaria worldwide in 2016 (range 149–303 million). The African Region accounted for most global cases of malaria (88%), followed by the South-East Asia Region (10%) and the Eastern Mediterranean Region (2%). In 2016, there were an estimated 438 000 malaria deaths (range 236 000–635 000) worldwide. Most of these deaths occurred in the African Region (90%), followed by the South-East Asia Region (7%) and the Eastern Mediterranean Region (2%). Between 2000 and 2015, malaria incidence rates (new malaria cases) fell by 37% globally, and by 42% in Africa. During this same period, malaria mortality rates fell by 60% globally and by 66% in the African Region. Other regions have achieved impressive reductions in their malaria burden. Since 2000, the malaria mortality rate declined by 72% in the Region of the Americas, by 65% in the Western Pacific Region, by 64% in the Eastern Mediterranean Region, and by 49% in the South-East Asia Region. For the first time, the European Region reported zero indigenous cases of malaria in 2015.Children under five are particularly susceptible to malaria illness, infection and death. In 2015, malaria killed an estimated 306 000 under-fives globally, including 292 000 children in the African Region. Between 2000 and 2015, the mortality rate among children under five fell by 65% worldwide and by 71% in Africa (WHO, 2016)

2.1.4 Country-Level Trends

Since 2000, there has been a significant increase in the number of countries that have moved towards malaria elimination. Of the 106 countries with ongoing malaria transmission in 2000, 57 achieved reductions in new malaria cases of atleast 75% by 2015. Eighteen countries reduced

their malaria cases by 50-75%. In 2015, 33 countries reported fewer than 1000 cases of malaria. In 2014, 16 countries reported zero indigenous cases of the disease: Argentina, Armenia, Azerbaijan, Costa Rica, Iraq, Georgia, Kyrgyzstan, Morocco, Oman, Paraguay, Sri Lanka, Tajikistan, Turkey, Turkmenistan, United Arab Emirates and Uzbekistan. In 2015, the global burden of malaria remained heavily concentrated in 15 countries, mainly in Africa. Together, these countries account for an estimated 80% of global malaria cases and 78% of deaths. Since 2000, progress in reducing malaria incidence in these high burden countries (32%) has lagged behind that of other countries globally (53%) (WHO, 2015).

2.1.5 Malaria in Africa

About 90% of all malaria deaths in the world today occur in Africa south of the Sahara. It is because the most effective malaria vector – the mosquito Anopheles gambiae – is the most widespread in Africa and the most difficult to control. An estimated one million people in Africa die from malaria each year and most of these are children under 5 years old (Malaria affects the lives of almost all people living in the area of Africa defined by the southern fringes of the Sahara Desert in the north, and a latitude of about 28° in the south. Most people at risk of the disease live in areas of relatively stable malaria transmission – infection is common and occurs with sufficient frequency that some level of immunity develops (Fujiokan and Aikawa, 2002) A smaller proportion of people live in areas where risk of malaria is more seasonal and less predictable, because of either altitude or rainfall patterns. In areas of stable malaria transmission, very young children and pregnant women are the population groups at highest risk for malaria morbidity and mortality (Desowitz, 1991).

Most children experience their first malaria infections the first year or two of life, when they have not yet acquired adequate clinical immunity – which makes these early years particularly

dangerous. Ninety percent of all malaria deaths in Africa occur in young children. Adult women in areas of stable transmission have a high level of immunity, but this is impaired especially in the first pregnancy, with the result that risk of infection increases. Malaria has been well controlled or eliminated in the five northernmost African countries, Algeria, Egypt, Libyan Arab Jamahiriya, Morocco, and Tunisia (Desowitz,1991).

2.1.6 Control of Malaria

During the past decade, large increases in funding have supported the scale-up of life-saving interventions for malaria control, contributing to substantial reductions in malaria morbidity and mortality. WHO estimates that between 2000 and 2010, global malaria incidence decreased by 17% and malaria-specific mortality rates by 26% (WHO, 2011). Although most investments and efforts have been directed towards high-burden countries impressive accomplishments have been made in malaria-eliminating countries including in southern Africa, Mesoamerica, Central Asia, and the Asia-Pacific region (Cotter *et al.,* 2013).

In the past 5 years, more countries have been certified as malaria free (Armenia, Morocco, Turkmenistan, and the United Arab Emirates) than in the previous 25 years combined. Malaria-eliminating countries have contributed substantially to the reduction of the global malaria burden over the past decade. The number of reported annual malaria cases for the 34 malaria-eliminating countries has decreased by 85%, from 1.5 million in 2000 to 232 000 in 2010 (WHO, 2011). In the same period, 25 of 34 malaria eliminating countries reduced total malaria cases by more than 70% with 17 countries reporting a greater than 90% reduction. Specifically, malaria-eliminating countries reduced their total caseload by 79% in the Asia- Pacific region, 86% in Latin America, 92% in sub- Saharan Africa, and 96% in the Middle East, Europe and central Asia. These successes have been driven by several factors, including increased funding, effective vector

control, strengthening of health systems, improved case management with more effective treatment regimens and improved case reporting and surveillance.

At the same time, gross domestic product per head in the 34 malaria eliminating countries increased by an average of 3.5% per year between 2000 and 2010, possibly creating a less favorable environment for transmission through urbanization and improved housing. These countries have invested heavily in malaria control and do not consider indefinite sustaining of malaria control to be an option. The envision of malaria elimination as the long-term goal that would protect their investments from emerging parasite and vector resistance and waning political and financial commitment (Chiyaka, 2013). In the Greater Mekong subregion (comprising Burma, Cambodia, Laos, Thailand, Vietnam, and the Yunnan Province of China), where artemisinin resistance has been documented (Dondorp, 2009), the response has been to move rapidly towards regional elimination. To achieve this aim, these countries need to address the same challenge that all the malaria-eliminating countries face: to attack the remaining parasite reservoirs, albeit with restricted choices of antimalarial drugs.

In malaria-eliminating settings, remaining parasite reservoirs are increasingly clustered in small geographical areas-so-called hotspots (Bousema, 2012). Cases are more clustered demographically into subpopulations with shared social, behavioural, and geographical risk characteristics referred to as hot populations or hot-pops. Within eliminating countries, an increasing proportion of cases are imported and outside sub-Saharan Africa, the proportions of all cases caused by *Plasmodium vivax* are rising (Feachem, 2010). In many malaria-eliminating settings, imported malaria is the sole or main threat to achievement and maintenance of elimination with greatest risk for countries neighbouring high-endemic areas (Chuquiyauri *et al.,* 2011; Martens and Hall, 2000).

In Saudi Arabia, for example, malaria cases decreased substantially between 1999 and 2010 and the proportion that was imported increased from 23% to 99%. Movement of people around the world can cause the disease to spread to non-endemic or previously eliminated areas; With the ever-increasing movement of people around the world, more instances of malaria reintroduction to receptive malaria-free areas have been documented (Cohen *et al.,* 2012). For example, China has eliminated *Plasmodium falciparum* from large parts of the country but with more Chinese nationals returning from work in sub-Saharan Africa, the country faces increasing rates of imported *P. falciparum* malaria.

Importation of malaria between islands in the Philippines, Solomon Islands, and Vanuatu is a constant threat as these countries pursue malaria elimination island-by-island. Knowledge of the dynamics of population migration both domestic, international and cross-border malaria transmission is crucial for development of appropriate surveillance and response systems. In high-endemic countries, particularly in sub-Saharan Africa, the focus of malaria control has understandably been on *P. falciparum*. However, outside sub-Saharan Africa, as malaria is controlled, the relative burden due to non-*falciparum* species increases and different challenges arise. In many countries where *P. falciparum* has been successfully eliminated, such as all malaria-endemic countries in Europe and central Asia, Argentina, Belize, Mexico, and large parts of China, *P. vivax* is the remaining challenge (Gething *et al.,* 2012) with increasing evidence that *P. vivax* infection causes substantial morbidity and mortality (Baird, 2013; Price *et al.,* 2007).

In countries with both *P. falciparum* and *P. vivax*, the ultimate challenge for elimination will be *P. vivax*. 26 of the 34 malaria eliminating countries (76%) have a malaria burden mainly due to *P vivax* (Feachem, 2010). Further, the risk of life threatening haemolysis in patients with glucose-

6-phosphate dehydrogenase (G6PD) deficiency, a common inherited blood disorder in malaria-endemic areas, causes health-care providers to hesitate to use the drug. A reliable point-of-care test to detect G6PD deficiency is not available (Mueller, 2009). *P. malariae* and *P. ovale* are less prioritised than *P. vivax* and *P. falciparum* in malaria control and elimination. Their true burdens are largely unknown because identification by microscopy or rapid diagnostic test (RDT) is not reliable. Similar to *P. vivax*, detection of *P. ovale* infection is also a challenge because it has a dormant liver stage. PCR-based testing in African and Asian settings shows a higher proportion of both *P. malariae* and *P. ovale* infections than was previously thought. *Plasmodium knowlesi*, which has a macaque monkey reservoir and has been reported in Borneo and in other parts of southeast Asia, can cause severe disease in human beings (Cotter *et al.,* 2013). The burden and distribution of *P. knowlesi* is not well defined because it is frequently misdiagnosed by microscopy as other species-most often *P. malariae* (Cox-singh and Singh, 2008). Surveillance based on molecular testing is limited. As burdens of *P. falciparum* decreases, malaria eliminating countries will need new strategies to diagnose, treat and interrupt the transmission of non-*falciparum* malaria.

Although microscopy and RDTs are the standard ways to diagnose malaria at health facilities, new and more sensitive methods to screen populations to identify low density subpatent infections are needed. Ideally, these new diagnostic tests will detect all plasmodia species infections at low density and be high throughput, lowcost and delivered at the point of care. Loop-attenuated isothermal amplification is the method that most closely matches this target profile since it is lower cost and more field-ready than are other nucleic acid tests. Although microscopy and RDTs continue to be used for screening, use of high throughput nucleic acid tests using pooling techniques can assure quality and identify missed infections, albeit on a

delayed timescale. Use of serology to measure past exposure to malaria could be a valuable means to identify at-risk populations, especially in low-transmission settings where the possibility of detection of current infection is low. Although methods have been established, no strategy for the incorporation of serology has been validated for malaria control or elimination. In areas where malaria has been eliminated, serology to detect exposure to the bite of anopheles mosquitoes could be used to indicate potential risk for reintroduction and support decisions on when to stop or restart vector control measures (Cotter *et al.,* 2013).

Mass drug administration (MDA) is the main method for control and elimination of many parasitic diseases, including lymphatic filariasis, onchocerciasis and schistosomiasis (Hotez, 2009). Although MDA for malaria has been widely used in China and Russia, little evidence of its effect has been collated and guidelines for its implementation do not exist. MDA is likely to be most effective during the lowest transmission season, with the aim to reduce or interrupt malaria transmission. Several key challenges need to be addressed for MDA. The optimum combination of drugs has not yet been determined but should include those that will affect the sexual (and liver) stages of malaria parasites, a formulation which would probably contain an artemisinin and an 8-aminoquinoline (Gosling *et al.,* 2011).

Clear identification of geographically or demographically defined at-risk populations will affect the design of an MDA strategy. Adequate resources supported by political commitment should be in place to interrupt transmission, because multiple rounds of MDA might be needed over several years in combination with other control measures (Okell *et al.,* 2011). Pilot projects with well designed monitoring and evaluation structures measuring adverse events, population acceptability and transmission reduction would support the progress and adoption of MDA as a

more widespread intervention. Identification of at-risk populations and the most effective methods to target them is crucial in an elimination setting.

Traditional vector control interventions, such as insecticide-treated nets and indoor residual spraying, protect the household but are less effective for individuals who are away from their homes during the peak times of vector feeding. In these circumstances, topical repellents, such as N,N-diethyl-3-methylbenzamide (DEET), botanicals, citronella, picaridin and olfactory binding proteins could be viable methods to protect these groups. DEET, which has been used for more than 60 years is effective against mosquitoes but has shown little effect on malaria prevention. Results of evaluations of DEET-based soap in Pakistan and a plant-based repellant in Bolivia showed significant reductions in *P. falciparum* and *P. vivax*, respectively. Textiles treated with longlasting insecticide that retains effectiveness for 70 washes and microencapsulated citronella oils to treat cotton textiles,can be low-cost, simple, adaptable, and scalable approaches to malaria prevention, if proven efficacious (Specos *et al.,* 2010).

To support targeted interventions, improved understanding of the at-risk population is needed. In control settings such factors can be established through nationally representative cross-sectional surveys, such as malaria indicator surveys. However, in areas where transmission is very low and malaria infections are rare, these surveys are unlikely to adequately detect cases or identify risk factors and are expensive (Hsiang *et al.,* 2012). Use of methods to support programmes to undertake case-control studies would provide crucial data for malaria risk factors and be of substantial value. Interventions that reduce the receptivity of an area to transmission could assist in the achievement and maintenance of elimination, as in the southeastern USA in the 1920s and 1930s (Humphrey, 2001).

The protective effects of housing structure improvements such as house screening, closing of eaves, and ceiling installation have been documented since the 19th century (Lindsay *et al.,* 2002). Despite potential higher up-front costs of such structural interventions, they are likely to be more cost effective over time because of their permanence and reduced reliance on individual behaviours, which is particularly important since user-driven malaria interventions such as insecticide-treated bednets are difficult to sustain when the perceived risk of malaria decreases.

Since the inception of the Malaria Vaccine Initiative in 1999, the goal of a malaria vaccine has been to save lives in the highest risk groups: young children and pregnant women. This goal remains important for high burden countries. However, in elimination settings, the use of a malaria vaccine that targets at-risk groups should be considered with the objective of transmission interruption. For example, in a seasonal setting, if the vaccine could induce enough immunity to reduce the basic reproductive rate to less than one in the population at risk for the duration of the malaria season and be administered in conjunction with other control measures, it might interrupt transmission. RTS,S, the only vaccine currently in Phase 3 clinical trials, does have high efficacy over a short duration, and might be useful for this purpose. Generally, vaccines that address transmission are being sought either through targeting of sporozoites or the sexual stages of both *P. falciparum* and *P. vivax*. The most promising vaccine candidates are in phase 2a studies (Vinnice *et al.,* 2012). Further investigation is needed of the role of an efficacious vaccine to target at-risk populations in elimination settings, with focus on transmission interruption.

2.1.7 Insecticide and Drug Resistanceof Malaria

Plasmodium falciparum has developed resistance to most available and affordable drugs. Chloroquine (CQ) resistance was first documented in Southeast Asia in the late 1950s and had spread to Africa by the end of the 1970s. (Wellems and plowe., 2001). Sulphadoxine–pyrimethamine (SP) has been used as a replacement for CQ but its effectiveness is now also seriously impaired by resistance. Despite this, many countries in Africa still rely on CQ or SP, or combinations of the two, as standard first-line treatment. Quinine is reserved for cases of treatment failure and for severe malaria, but this is a difficult drug to use and is also relatively expensive at about $1 per treatment course. Artemisinin-based combination therapies (ACTs) were highly effective against *P. falciparum*, the most prevalent and lethal malaria parasite affecting humans. Artemisinin derivatives are highly potent, rapidly eliminated antimalarial drugs with a broad stage specificity of action. They clear parasitemia more rapidly than all other currently available antimalarial agents. Globally, the number of ACT treatment courses procured from manufacturers increased from 11 million in 2005 to 337 million in 2014. The African Region accounted for most (98%) manufacturer deliveries of ACTs in 2014. Malaria infection during pregnancy carries substantial risks for the mother, her fetus and the newborn child. In Africa, the proportion of women who receive intermittent preventive treatment in pregnancy (IPTp) for malaria has been increasing over time, but levels remain below national targets (WHO, 2015). In the 1990s, resistance to available antimalarial drugs such as chloroquine and sulfadoxine–pyrimethamine worsened across areas of the world where malaria is endemic. As a direct consequence, morbidity and mortality associated with malaria increased, especially among African children, who account for most deaths from malaria (Snow *et al.*, 2001).

Artemisinin resistance has occurred as a consequence of several factors: poor treatment practices, inadequate patient adherence to prescribed antimalarial regimens, and the widespread availability of oral artemisinin-based monotherapies and substandard forms of the drug. The geographic scope of the problem could widen quickly and have dire public health consequences: the spread or independent emergence of artemisinin resistance in other parts of the world could pose a major health security risk as no alternative antimalarial medicine is available at present with the same level of efficacy and tolerability as ACTs (WHO, 2013) As of July 2016, artemisinin resistance has been confirmed in 5 countries of the Greater Mekong subregion (GMS): Cambodia, the Lao People's Democratic Republic, Myanmar, Thailand and Viet Nam. In the large majority of sites, patients with artemisinin-resistant parasites still recover after treatment, provided that they are treated with an ACT containing an effective partner drug. However, along the Cambodia-Thailand border, *P. falciparum* has become resistant to almost all available antimalarial medicines. There is a real risk that multidrug resistance will soon emerge in other parts of the subregion as well. The World Health Organization (WHO) recommends that treatment policy should be changed once clinical failure rates reach 15% (WHO, 2001). A moment's reflection would suggest to the clinician that acceptance of up to a 15% failure rate, in a disease that can be fatal, would be unacceptable in most walks of life. Be that as it may, most countries in sub-Saharan Africa have long reached this point and must now decide on their next therapy. The challenge for malaria treatment policy-makers is to find an affordable and effective treatment to which the parasite will not rapidly become resistant (David and Peter, 2004).

Mosquito resistance to insecticides is another growing concern. Since 2010, 60 of the 78 countries that monitor insecticide resistance have reported mosquito resistance to at least one

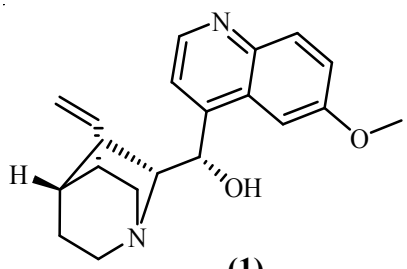
insecticide used in nets and indoor spraying; of these, 49 reported resistance to two or more insecticide classes (Heggenhougen *et al.*, 2003).

2.1.8 Anti-Malaria Drug

Anti-malaria drugs are designed either for preventive or curative purposes. The drugs act by inhibiting the heme polymerase enzymes which eventually leads to the accumulation of cytotoxic heme and poison the parasite (White, 2004). Example of these drugs include

Quinine (1)

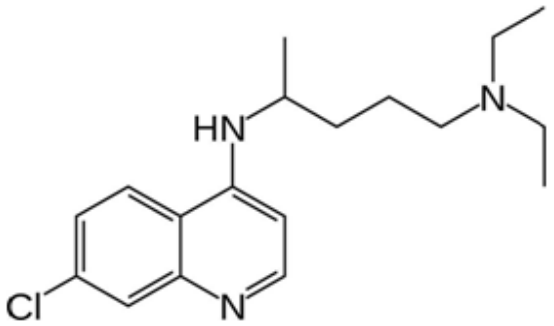
Quinine has a long history, the discovery of the compound is traced from Peru cinchona tree and the potential uses of its bark. Collection of its derivatives are still frequently used in the prevention and treatment of malaria (White, 2004).



(1)

Chloroquine (2)

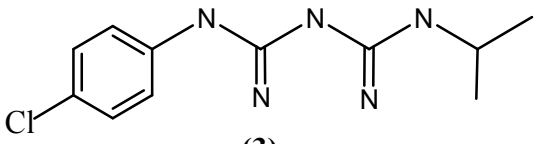
This is a 4-aminoquinoline compound with a complicated and still unclear mechanism of action. It controls the conversion of toxic heme to hemozoin by inhibiting the biocrystallization of hemozoin, thus poisoning the parasite through excess levels of toxicity (White, 2004).



(2)

Proguanil (3)

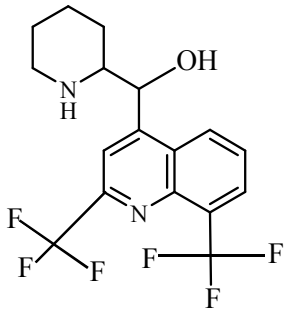
This is a biguanide, a synthetic derivative of pyrimidine which is active against several protozoal species. It is metabolized by the liver to cycloguanil which has potent antimalarial activity. Cycloguanil appears to act by inhibition of *Plasmodial* dihydrofolate reductase-thymidine synthetase and there by interfering with folate metabolism and DNA synthesis (White, 2004).



(3)

Mefloquine (4)

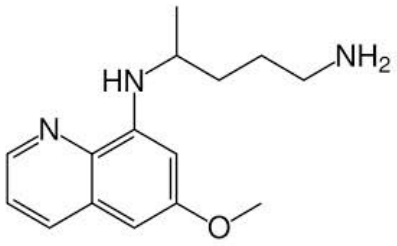
This is a phospholipid-interacting antimalarial drug which acts as a blood schizoticide. It is active against the erythrocytic stages of *Plasmodium* specie. However the drug has no effect against the exoerythrocytic (hepatic) stages of parasite (White, 2004).



(4)

Primaquine (5)

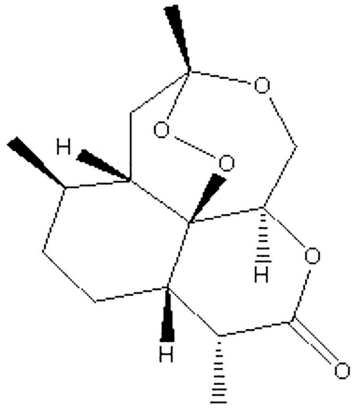
This is a highly active 8-aminoquinoline that is used in treating all types of malaria infection. It is known to cure both replasing malaria infections and acute cases. It acts by interfering with a part of the parasite (mitochondrion) that is responsible for supplying it with energy (White, 2004).



(5)

Artemisinine (6)

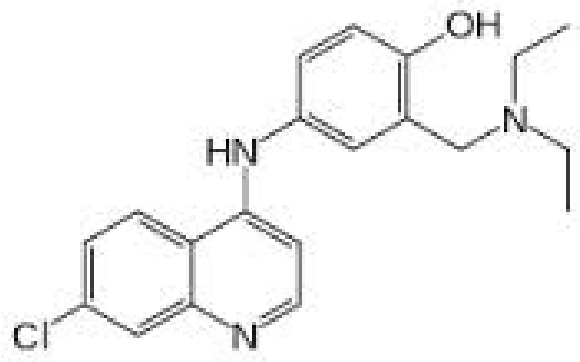
This is a sesquiterpene lactone isolated from Artemisia annua, with a chemically rare peroxide bridge linkage. The mechanism of action of artemisinin and its derivatives results in the conversion of the drug to active metabolite dihydroartemisinin that inhibits the endoplasmic reticulum laicium at phase encoded by *Plasmodiumfalciparum* (White, 2004).



(6)

Amodiaquine (7)

This is a pro-drug for the active antimalarial metabolite desethylamodiaquine. It is similar in structure and mechanism of action to chloroquine. It has been shown to be more effective on chloroquine resistant *Plasmodium falciparum* malaria infection. (White, 2004).



(7)

One promising approach in malaria research is the investigation of plants (Rasaonaivo *et al.,* 1999; Madurera *et al.,* 2002). A number of plants are already reported for the treatment of `malaria. Research reports showed quite a number of anti-plasmodialcompounds isolated from medicinal plants (Joanne *et al.,* 2009).

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2.1.9 Medicinal Plants in the Treatment of Malaria

Traditional herbal medicines have been used to treat malaria for thousands of years in various parts of the world. The first antimalarial drug used in the Occident was extracted from the bark of the Cinchona (Rubiaceae) species, the alkaloid quinine, still largely used. Infusions of the plant bark were used to treat human malaria as early as 1632 (Baird *et al.,* 1996). Years later quinine was isolated and characterized (Saxena *et al.,* 2003), thus becoming the oldest and most important antimalarial drug. Another ancient medicinal plant of millenium use in the West is Artemisia annua, rediscovered in China in the seventies as an important source of the antimalarial artemisinin (Bruce-Chwatt, 1982; Klayman, 1985). Artemisinin-combined therapies (ACT) were formally adopted as first-line treatment of uncomplicated malaria in Nigeria from 2005 onwards (Mokuolu *et al.,* 2007). However, ACT use is limited due to its high costs, limited production of artemisinin derivatives to Good Manufacturing Practices (GMP) standards and toxicity (Haynes, 2001). Neither the Cinchona plants nor Artemisia annua, from which the most

potent drugs (quinine and artemisinin) were isolated, are indigenous to sub-Saharan Africa. Tropical rainforest plants are known to have higher concentrations of natural chemical defenses a`nd a greater diversity than plants from any other biome, thus they are potential sources of new medicines. Nigeria has rich flora diversity and many of the plant species are used by some indigenous people for medicinal purposes. A larger number of medicinal plants are used to treat malaria in the Southern part of the country where rain forests exist and originate a humid tropical climate, with ideal czzonditions for persistent malaria transmission all year round.

Some plant species used are Morinda lucida (Rubiaceae), largely used in malaria treatment in Nigeria, is a tree of 9–18m in height, bearing a dense crown of slender crooked branches (Avwioro *et al.,* 2005). The wood is moderately coarse, open grained with a tendency to spiral, though not warping nor altering, fairly hard and of medium weight. The aerial parts, stem bark or root bark of Morinda lucida are widely used in West Africa to treat malaria and other tropical diseases. A seasonal variation in its antimalarial activity has been reported (Makinde *et al.,* 1994). Nauclea latifolia (Rubiaceae) is a small spreading tree, essentially a savannah plant, used as aqueous decoction of the root bark against malaria. Several other plants are used for malaria treatment in Nigeria, like *Quassia amara* and *Quassia undulata* (Simaroubaceae), largely used in the southwestern part of the country (Phillipson and Wright, 1991). *Quassia amara,* called bitterwood tree, with 2–6m in height, has the highest antimalarial reputation for curative and preventive purposes in the Simaroubaceae family. *Enantia chlorantha* (Annonaceae) is an ornamental tree, of up to 30m in height, with dense foliage and spreading crown. Its stem bark is used against fever/malaria by traditional medicine practitioners in the forest regions. *Carica papaya* (Caricaceae), commonly referred to as pawpaw, is widely grown in the tropics for its edible fruit and also used as a weak decoction of its leaves against malaria. It is a small tree with

a weak soft-wooden stem which is usually unbranched and hollow. Fagara zanthoxyloides (Rutaceae) has its root widely used as chewing stick in Nigeria and West Africa in general (Odebiyi and Sofowora, 1979). The aqueous extract of the root is used for malaria treatment by the indigenous people. *Spathodea campanulata* (Bignoniaceae), popularly known as African tulip tree is native to tropical Africa, though it has now been adapted to other tropical regions around the world, mostly because of its ornamental value. It is used in southwestern Nigeria for malaria treatment by drinking the decoction of its stem bark. Most of these antimalarial plants are used in form of monotherapy, and only a few plants are taken together in combined therapies. An example is the multi-herbal extract referred to as 'Agbo-Iba' madeup of *Cajanus cajan* (pigeon pea) leaf, Euphorbia lateriflora leaf, *Mangifera indica* leaf and bark, Cassa alata leaf, *Cymbopogon giganteus* leaf, *Nauclea latifolia* leaf, and Uvaria chamae bark. Another multi herbal combination is the mixture of *Carica papaya* leaves, *Cymbopogon citratus* leaves, *Anacardium occidentale* leaves and *Azadirachta indica* leaves used in 'steam therapy', in which the patients are covered with a thick blanket and made to inhale the vapour from the cooking pot (Nwabuisi, 2002).

2.2 *senna occidentalis*

Sennaoccidentalis is widely distributed and very commonly used plant. *Sennaoccidentalis*, which is commonly called '*Dora rai'* in Hausa, '*Akidi ogbara'* in Igbo, '*Abo rere'* in Yoruba and 'Coffee senna' in English (Alhassan *et al.,* 2017). It contained many chemical groups including alkaloids, cyanides, phenolics, proteins, tannins, steroids, tannins, flavonoids, anthroquinone, saponins, terpenes, resins, balsams, amino acids, carbohydrates, sugars and cardiac glycosides (Vijay`alakshmi *et al.,* 2013). *Sennaoccidentalis* exerted many pharmacological effects included antimicrobial, anthelmintic, insecticidal, antioxidant, antianxiety, antidepressant, antimutogenic

antidiabetic, and wound healing, hepatoprotective, Reno protective, sun protective, and smooth muscles relaxation, immune-modulating, antiinflammatory, analgesic, antipyretic and other effects. The present review will highlight the chemical constituents and the pharmacological and therapeutic effects of *Sennaoccidentalis*. Synonyms: *Senna occidentalis*.

2.2.1 Taxonomic Classification

Kingdom: Plantae, Phylum: Spermatophyta, Subphylum: Angiospermae, Class: Dicotyledonae, Order: Fabales, Family: Fabaceae, Subfamily: Caesalpinioideae, Genus: *Senna*, Species:*Senna occidentalis (*Al-Snafi, 2015).

2.2.2 Distribution

S. occidentalis is a spiny herb grows all over in tropical countries inshade as well as under open condition. Generally found up to an altitude of 1,000 m in northern part of Nigeria and wild throughout the plains on waste lands or in the coastal areas. It is also found in deltaic region of western, eastern and southern Nigeria found particularly in west Africa Nigeria in particular and seacoast throughout the hotter parts of India, Burma and Sri Lanka and it is also grown as an ornamental plant.

2.2.3 Botanical description

Leaves are evergreen, lanceolate, compound, glossy leaf surface, deep tap root system, stem is hard and woody, Dicot seed type with characteristic odour and bitter taste. Flowers are yellow in colour with 1 to 2 cm diameter arranged in raceme type inflorescence, axillary and also forming terminal, bracts are caduceus. Fruits are flat pods, 10-12 cm long with 10-30 seeds. Areolate seeds are pointed at end and blunt at the other.

2.2.4 Major chemical constituents

The main plant chemicals in *S.occidentalis* include: achrosin, aloe-emodin, emodin, anthraquinones, anthrones, apigenin, aurantiobtusin, campesterol, cassiollin, chryso obtusin, chrysophanic acid, chrysarobin, chrysophanol4, chrysoeriol and essential oils (Shankar, 2003). funiculosin, galactopyranosyl, helminthosporine (Kudav and Kulkarni, 1974), islandicine, kaempferol, lignoceric acid, linoleic acid, linolenic acid, mannitol, mannopyranosyl, matteucinol, obtusifolin, obtusin, oleic acid, physcion , quercetin, rhamnosides, rhein, rubrofusarin, sitosterols, tannins, and xanthorine (Chukwujekwu *et al.,* 2006). The study of phytochemicals of *S.occidentalis* reveals that the nature and amount of phytochemicals vary according to climate. For example stems, leaves and the root bark of the plant from Ivory Coast, Africa contain small amount of saponins, no alkaloids, sterols, triterpenes, quinines, tannins and flavonoids. However, a large amount of alkaloids were found in the stem, leaves and fruits from Ethiopia (Smolenski *et al.,* 1975).

2.2.5 Traditional uses

S. occidentalis is regarded as 'Edible weeds of agriculture or 'Famine food'. Its infusion is given against the white discharge. In Mali, *S.occidentalis* is used as ingredient in a malarial formulation based on a traditional recipe comprising leaf of *C. occidentalis* leaves of Lippia chevalieri and flower heads of Spilanthes oleraces (Willcox *et al.,* 2012). Decoction of *S.occidentalis* roots with black pepper isuseful in filarial (Arvind *et al.,* 2007). In the Malyagiri hills, a decoction is made from 15 leaves each of *S.occidentalis*, Glycosmis pentaphylla and Vitex negundo and used for bathing the new born baby to make the baby immune to skin diseases (Yadav *et al.,* 2010). According to 'Bhavaprakasa', Kasamarda (*S.occidentalis*) is used in constipation, and is stated that leaves, roots and seeds are useful as purgative

(Warrier and Nambiar ,1994). In folklore medicine, seed powder (half a tea spoon) is used to cure fever while two table spoons of leaf juice mixed with honey cures cough. For intestinal gas half a cup of leaf decoction is taken twice daily and paste of leaf is applied for skin diseases.

2.2.6 Medicinal properties of *Sennaoccidentalis*

Phytochemical screening of the plant showed the presence of carbohydrates, saponins, sterols, flavonoids, resins, alkaloids, terpenes, anthraquinones, glycoside and balsam. Presence of these metabolites strongly concluded the great potential of the plant as a source of phytomedicines (Al-Snafi *et al*., 2015). As the flavonoids and resins are present, it might be responsible for its anti-inflammatory properties. Chinese folkloric medicine contains flavonoids which has anti-inflammatory effect on both acute and chronic inflammation. Alkaloids are known for decreasing blood pressure, balancing the nervous system in case of mental illness and antimalarial properties. Tannins help in wound healing and anti-parasitic. Presence of terpenes suggests possessing anti-tumor and anti-viral properties. Eudesmane sesquiterpenes have been reported to contain antibacterial properties. Saponins are believed to have antioxidant, anti-cancer, anti-inflammatory, and anti-viral properties. The anthraquinones, emodin and chrysophanone have been reported to possess wound healing properties. Other compounds reported in literature include, 1,8-dihydroxyl-2-methyl anthraquinone, 1,4,5- trihydroxy-3-methyl-7-methoxy anthraquinone, *Senna occidentalis* A, B and C, which are C-glycosides, achrosine, anthrones, apigenin, aurantiobtusin, campstool, cassiollin, chryso-obtusin, chrysophanic acid, chrysarobin, chrysoeriol, essential oils, funiculosin, galactopyranosyl, helminthosporin, islandicin, kaempferol, lignoceric acid, linoleic acid, linolenic acid, mannitol, mannopyranosyl, matteucinol, obtusifolin, obtusin, oleic acid, physcion, quercetin, rhamnosides, rhein, rubrofusarin, sitosterols, and xanthorin (Al-Snafi *et al*., 2015).

2.2.6.1 Antimicrobial activity

A study was carried on *Sennaoccidentalis* antimicrobial properties (Willcox *et al.,* 2012). Test was conducted with four different extracts such as methanol, aqueous, benzene, petroleum ether and chloroform extract. Among which methanol extract showed positive against *P. aeruginosa, K. pneumoniae, P. mirabilis, E. coli, S. aureus and S. epidermidis; aqueous extract was effective against P. vulgaris, K. pneumoniae and P. aeruginosa*; benzene and petroleum ether extracts was active against P. mirabilis and E. coli; chloroform extract was found to be very inactive against all tested strains. Another study (Arvind *and* shamshun, 2007) reported maximum activity against *Salmonella typhi* and minimum with *Shigella spp*.

A report by (Yadav *et al.,* 2010) with *Senna occidentalis* flower extract showed maximum inhibition against Klebsiella pneumonia and no activity against *Staphylococcus aureus, Streptococcus pneumoniae,* and *Pseudomonas aeruginosa*. Thus the flower extract of *Sennaoccidentalis* can be used to treat Klebsiella associated ailment such as pneumonia, bronchitis and other diseases known to cause by K. pneumonia. A report by (Warrier and Nambiar, (1994) states that the *E. coli* was sensitive to methanol, hexane, chloroform and aqueous extract of leaves of *S.occidentalis*. Similarly, Jain and his coworkers (Bhattacharyya *et al.,* 2003) observed that the metabolite rich fraction of (anthraquinones) leaves, pods, flowers and callus were effective against *E. coli.* Yet other study showed that the petroleum ether and ethanolic extract of leaves of *S.occidentalis* was active against E. coli. with chloroform and aqueous extract the inhibition was not observed against E. coli. Based on the experiments it can clearly say that changes in the activities of plant extracts might be due to spatial and temporal variations. *P.aeruginosa* showing multidrug resistance is highly challenging to treat by conventional antibiotics. A study (Kolhapure and Miltra, 2004) tested the efficiency of leaf

extract of *S.occidentalis* against the growth of *P.aeruginosa* and found that the microbial growth was highly inhibited. And the crude extracts was effective on some microbes such as *Staphylococcus aureus, Escherichia coli, Bacillus subtilis and Candida albicans* which was a common causative agent of urinary tract infection and diarrhea diseases (Tona *et al.,* 2004). As this plant has potential antimicrobial activity but invivo studies with the extract should be carried out to confirm that the zone of inhibition is not only by the sensitivity of the microbes also the concentration is highly essential when using for treatment.

2.2.6.2 Antioxidant activity/hepatoprotective activity

The aqueous–ethanolic extract of leaves of *S.occidentalis* was tested for hepatoprotective activity on liver damage in rat which was induced by paracetamol and ethyl alcohol by monitoring serum transaminase, alkaline phosphatase, serum cholesterol, serum total lipids and histopathological alterations. The result stated that the leaf extract had shown significant hepatoprotective activity (Sharma *et al.,* 2000). Some other observations had found that the seed extracts of *S.occidentalis* limits the DNA degradation caused by iron (II) –driven Fenton reaction. It is notable that inhibition of DNA damage may be due to their capability of strong ferrous ion chelation. Further, study proposed that the scavenging activity towards free radicals might be the reason. *S.occidentalis* is an ingredient in Himoliv, a polyherbal ayurvedic formulation. It is also proved that it prevents the carbon tetra chloride induced hepatotoxicity in rats (Gupta *et al.,* 2003). Based on the observation they suggested that Himoliv increases the protective enzymes superoxide dismutase (SOD) and catalase in liver homogenate of rats (Mukherjee *et al.,* 2002). It is also present in other polyherbal formulation Liv.52 tablet and syrup extensively used for Hepatitis A (HA). For the preparation of this syrup, other plants

included *Capparis spinosa, Cichorium intybus, Solanum nigrum, Terminalia arjuna, Achillea millefolium* and *Tamarix gallica* etc along with *S.occidentalis* are present.

A study with 50 clinical samples over 30years with 4490 patients was performed to identify the efficacy with short and long term safety of Liv.52 in Hepatitis A (Silva *et al.,* 2011). This study concluded that Liv.52 tablets and syrups are potential and safer for hepatitis A.

2.2.6.3 Larvicidal Activity

The larvicidal and pupicidal potential of *SennaOccidentalis* was analyzed in a study (Daniyan *et al.,* 2011) against the larvae of Anopheles Stephensi. The ethanol extract of *Senna* Occidentalis were found to be more effective against larva and pupa respectively. The smoke toxicity study was also conducted and identified that it was more effectiveagainst the *Anopheles stephensi*. Smoke exposed gravid females oviposited fewer eggs when compared to those that were not exposed. Yet another study (Jain *et al.,* 1998) reveal that seed oil creates increaszein mortality of *C. maculatus* eggs. Based on numerous trials with pure compounds suggested that fatty acids (linoleic, oleic and stearic) are responsible for *C. occidentalis* toxicity.

2.2.6.4 Anti-inflammatory activity

*Senna*occidentalis leaf powder was tested for anti-inflammatory activity and *Cardiospermum halicacabum* aerial parts with ethanol extract was assayed in male albino rats using carrageenan-induced rat paw edema. At 2000mg/kg dose the *S.occidentalis* was found to be active at maximum level and 500 mg/kg was found to be the minimal active dose for *C. halicacabum.* The efficiency was tested in cotton pellet granuloma assay and observed that the transudative, exudative and proliferative components of chronic inflammation were suppressed by these drugs. Lipid peroxide content and γ-glutamyl transpeptidase and phospholipase A2 activity in the exudates of cotton pellet granuloma was lowered with the usage of these

drugs. In normalized cotton pellet granulomatous rats, increased alkaline phosphatase activity with decreased A/G ratio of plasma were found after the treatment. *S.occidentalis* powder and *C. halicacabum* extract were able to stabilize the human erythrocyte membrane against hypotonicity-induced lysis. It is likely that these drugs may exert their anti-inflammatory activity by inhibition of phospholipase A2, resulting in the reduced availability of arachidonic acid, a precursor of prostaglandin biosynthesis and/or by stabilization of the lysosomal membrane system (Basri *et al.,* 2005).

2.2.6.5 Ant anxiety and Antidepressant activity

Around 5% of world's population was affected by anxiety and depression a widespread psychiatric disorder. Previously, plants and formulations were used to treat anxiety and depression over decades. A recent report has studied the antianxiety and antidepressant activity of ethanolic and aqueous extracts of *Sennaoccidentalis*leaves in rodents. Exposing the rats to unfamiliar aversion in different methods like elevated plus maze model and actophotometer antianxiety activity was tested. Less aversion fear elicits antianxiety activity. Antidepressant activity was analyzed by despair swim test and tail suspension test. Reduced immobility time elicits antidepressant activity. They conclude that ethanolic and aqueous extracts of *Senna occidentalis* leaves possess antianxiety and antidepressant activity. Ethanolic extract of *Sennaoccidentalis* leaves showing more significant activity over the aqueous extract (Rani *et al.,* 2010)

2.2.6.6 Analgesic and antipyretic activity

Sennaoccidentalis Linn screened for analgesic and antipyretic activity. Ethanol and water `extracts of *Sennaoccidentalis* leaves were screened in mice which was induced by acetic acid and tested for hot plate and tail immersion assay, and also in yeast induced pyrexia method in rats. The result found that the ethanol and water extracts of*Senna*

*occidentalis*possess antinociceptive and antipyretic properties. Highest inhibition dose was found to be as 300 mg/kg. The report clearly mentioned that both the ethanolic and water extracts of *Sennaoccidentalis* showed significant effect on pyrexia induced by yeast (Jafri *et al.,* 1999).

2.2.6.7 Antidiabetic activity

The aqueous extract of *S.occidentalis* was tested for antidiabetic activity and the study proved that there was a significant reduction in fasting blood glucose levels in the normal and alloxan i`nduced diabetic rats (Yadav *et., al* 2010) . Tested for other extracts include petroleum ether and chloroform extracts and concluded that activity from day 14 and activity from 7 days respectively. Specific variations were seen in serum lipid profiles (cholesterol and triglyceride), serum protein and changes in body weight by aqueous extract treated-diabetic animals, when compared with the diabetic control and normal animals.

2.2.6.8 Antimalarial activity

The *S. occidentalis* plant extract has a significant antimalarialactivity (Tona *et.,al* 2001). The ethanolic, dichloromethane andlyophilized aqueous extracts of *S. occidentalis* root bark wasevaluated for their antimalarial activity in vivo, in 4-day,suppressive assays against Plasmodium berghei ANKA in miceNo toxic effect or mortality was observed in micetreated, orally, with any of the extracts as a single dose, of500 mg/kg body weight, or as the same dose given twiceweekly for 4 weeks (to give a total dose of 4 g/kg). At doses of200 mg/kg, all the ethanolic and dichloromethane extractsproduced significant chemosuppressions of parasitemia of>60% for *S. occidentalis* root bark when administered orally.The *S. occidentalis* was active and cause 60% chemosuppression.It is also observed that the lyophilized aqueous extractwas less active than the corresponding ethanolic extract (Tona *et.,al* 2001)..Ethanol and chloroform

extract of the *S. occidentalis* leaveshave been found to have good antimalarial activity. Theseextracts produce more than 60% inhibition of the parasite growth in vitro at a concentration of 6 µg/ml(Tona *et.,al* 2004). .

2.2.6.9 Toxicological studies of *senna occidentalis*

The toxic effects of *S. occidentalis* in the case of animalswere found mainly on skeletal muscles, liver, kidney andheart. In animals the toxicity dose of beans varies from assmall as 0.05% to 0.5% of body weight. The acute liver andmuscle degeneration was chiefly observed in animals (Ohara *et., al* 1969).Signs of intoxication in the chicken were weight loss,weakness, diarrhea, hypothermia, occasionally ataxia, recumbency,and death. Gross lesions included paleness of skeletaland cardiac muscles and congestion of the liver (Bruere, 1943). Inanother study, signs of toxification were found in chickens asfocal swelling, fragmentation, and necrosis of myofibers ofthe semitendinosus muscle in histological sections.

Toxicological studies on liver mitochondria demonstratedlower phosphorylation ratios, respiratory control ratios, andrates of oxygen use in treated 3- to 4-week-old chicks (Graziano *et al.,* 1983). Seeds of *S. occidentalis* were found to be toxic in pigs as theydeveloped ataxia and other signs of neuromuscular dysfunctionwithin 6 or 8 weeks. Toxicological studies showedlethargy, weakness, recumbency, depression, and emaciationin rats when fed with 1%, 2%, and 4% of seeds (Colvin *et al.,*1986). The experiments showed the toxic effects of *S. occidentalis* onrabbits. The histopathological examination of rabbits revealedthat the heart and liver were the most affected organs withmyocardial necrosis and centrolobular degeneration. Theyalso found a reduction in cytochrome oxidase activity in theglycogenolytic fibers, together with muscle atrophy, confirmedby the morphometric studies (Tasake *et al.,* 2000). Many

outbreaks ofacute childhood illnesses with severe brain dysfunction(other than Japanese encephalitis (JE) due to consumptionof seeds of *S. occidentalis* occur in different parts of India. Theymay be at different times and at different places (Rao *et al.,* 2000).

The *S. occidentalis* poisoning in children seems to affectmainly three systems—hepatic, skeletal muscles and brain. *S. occidentalis* pods causes poisoning and results in fatalcoma in the children of Western Uttar Pradesh. Toxicity of theCassia beans is dose dependent. The consumption of 1–2 podsby a young child may not have any deleterious impact; a large'binge' can lead to serious disease and death (Vishatha, 2007). Leaves ofthe *S. occidentalis* plant have also been found to containtoxic phytochemicals that may be toxic to humans. A detailedstudy on brine shrimps for the investigation of toxicity ofMethanolic-chloroform extract of leaves of *S. occidentalis*revealed that this plant extract possesses a LC50 value at 99.5 µg/ml (Orech *et al.,* 2000). In another study, leaf extracts of the *S. occidentalis* plant have exhibited lethality on brine shrimps ata LC50 value of more than 1000 µg/ml . In further investigations, the aqueous leaf extract of this plant has beenfound to possess hypoproteinaemic effects and the levels ofthe enzymes alanine amino transferase, aspartate aminotransferase and alkaline phosphatase are significantly elevatedwhich show *S. occidentalis* leaves may be slightly toxic as aconcoction for liver ailments (Nuhu and Aliyu, 2008).

However, in contrast, theroot, leaves and stems were found to be toxic for cattle onlywhen large amounts are consumed but in rats toxicity of theleaves were observed at the dose of 12.5 g/kg body weight inrats (Nwudue and Ibrahim, 1980). (Arago *et al.,* 2009) studied the reproductive toxicity of the *S. occidentalis* plant extract on pregnant female rats. Inthis study, three groups of pregnant rats were treated orallyfrom the 1st to the 6th day (pre-implantation period) andfrom the

7th to the 14th day (organogenic period) of pregnancy, with doses of 250 and 500 mg/kg. They reported that there was no significant difference between the controland treated groups in terms of offspring/dam relationship,placenta and ovary weights etc. However, they observed the presence of dead fetuses in both doses of 250 and 500 mg/kgof *S. occidentalis*. (Badami *et al.,*2003) studied the reproductivetoxicity of an ethanolic extract of this plant along with Derris brevipes and Justicia simplex in rats and it was observed thatthe ethanolic extract possesses a more abortifacient typeeffect than the anti-implantation activity. The ethanolicextract also exhibited weak estrogenic activity when given alone and tested in immature ovariectomized female albinorats.

CHAPTER THREE

MATERIALS AND METHODS

3.1 MATERIALS

3.1.1 Equipment

All the equipment, apparatus and glasswares used in the research are listed in Appendix II.

3.1.2 Chemicals and reagents

All chemicals and reagents are listed in Appendix III.

3.1.3 Collection and Processing of*Senna occidentalis*leaves

Fresh Leaves of *senna occidentalis*leaves were collected from Bayero University Kano, Kano State and identification was done by a Botanist at Herbarium in the Department of Plant Biology, Bayero University Kano. A sample with accession number BUK/HAN/ 0073 was deposited at the herbarium of the Department.

3.2 METHODS

3.2.1 Extraction of *Senna occidentalis*leaf

Aqueous-Methanol extraction of the leaves was done according to the method described by Veeramuthu *et al.* (2006). Leaves of*Senna occidentalis*was air dried under shade and grounded using a grinding mill. The powdered leaf material weighing 100g was macerated with 1000 ml of 70 % aqueous methanol in an Erlenmeyer flask and placed on orbital shaker (Gallenkamp 5A-4131, England) at room temperature for 72 hours. The leaf extract was then filtered through cotton cloth and subsequently with a filter paper (12.5 cm size). The process was repeated until the leaves dark brown was exhausted and rotary evaporator was used to concentrate the extract. The concentrated crude extract was collected and stored in polythene bag.

3.2.2Fractionation of the Crude Aqueous Methanol Leaf Extract

The fractionation was done by partitioning the aqueous-methanol extract on the order of increasing polarity, starting with n-hexane (index 0.1), chloroform (index 4.1) and finally ethyl acetate (index 4.4).

3.2.3 Malaria Parasite Assay

3.2.3.1 Preparation of Test Solution

A stock solution (10,000µg/ml) was prepared by dissolving the extracts (20mg) obtained from *Senna occidentalis*leaves in dimethylsulphoxide (DMSO) (2ml). The following concentrations; were made by serial dilution as follows:

- 500µg/ml (0.05ml of stock solution + 0.95ml DMSO)
- 1000µg/ml (0.1ml of stock solution + 0.9ml DMSO)
- 2000µg/ml (0.2ml of stock solution + 0.8ml DMSO)
- 5000µg/ml (0.5ml of stock solution + 0.5ml DMSO)

3.2.3.2 Sourcing of Malaria Parasite

Malaria parasites of infected blood samples containing a parasiteamia of *Plasmodium*falciparum were collected from the Department of Haematology, Bayero University Clinic, Kano. The samples were received in K3-EDTA coated disposable plastic sample bottles with tightly fitted plastic corks, and transported to the Microbiology laboratory of Bayero University, Kano.

3.2.3.3 Determination of *Plasmodium falciparum* (Positive Blood Samples) by Thin Smear Method

The method adopted was as described by Decei and Lewis (1968). Using a clean capillary tube, a small drop of each blood sample was placed at the centre of a clean glass slide at least 2mm from one end. Smears were formed by moving the cover slip forward on each glass slide. The thin smears were immersed in methanol contained in a petri dish for about 15minutes. Geimsa's stain was dropped on each smear, and allowed to stay for about 10minutes. Excess stain was washed with clean tap water. The smears were dried in air by hanging the glass slides inverted in a rack. Each dried smear was microscopically observed under a high power objectives (x100) using oil immersion. An average parasitaemia was determined using the reading of 3 microscopic fields (Hanne *et al.,* 2002).

3.2.3.4 Separation of the Erythrocytes from the Blood Samples

Exactly 50% dextrose solution (0.5ml) was added to each of the blood samples (5ml) defibrinated, and then centrifuged at 2500rpm for 15minutes in a spectra merlin centrifugation machine. Supernatant layers were separated from the sediments. The latter was diluted with 0.5 ml normal saline (Dacie and Lewis, 1968) and centrifuged at 2500rpm for 10minutes again. The resulting supernatants were discarded. Samples with higher parasitaemia (above 5%) were diluted with fresh malaria parasite negative erythrocytes (Hanne *et al.,* 2002).

3.2.3.5 Preparation of Culture Medium for parasite cultivation

Blood sample (2ml) from a healthy rabbit were withdrawn using a disposable 5ml syringe (BD 205WG). The sample was defibrinated by allowing it to settle for atleast 45minutes (Lewis, and lewis 1968). The defibrinated blood sample was further centrifuged at 2500rpm for 10minutes. The supernatant layer was collected and sterilized. The separated sediment was centrifuged

further for about 5 minutes. The supernatant layer was added to the first one in a test tube. The sediments were discarded. The serum was supplemented with RPMI 1640 salt medium and sterilized 50 μ g/ml gentamicin sulphate (Trager, 1982). The composition of the RPMI 1640(Roswell Park Memorial institute medium) salt is as described below; KCl 5.37mM, NaCl 10.27mM, $MgSO_4$ 2.56mM, $NaHPO_4$ 17.73mM, $Ca(NO_3)_2$ 0.42mM, $NaHCO_3$ 2.5mM and glucose 11.0mM as demonstrated by (Devo *et al.,* 1985).

3.2.3.6 *In-Vitro* Assay of the Activity of the Extracts on *Plasmodium falciparum* Culture

A tested solution (0.1ml) of 500, 1000, 2000, and 5000µg/mland the culture medium (0.2ml) were added into a test tube containing 0.1 ml 0f 5% parasitaemia erythrocytes and mixed thoroughly. The sensitivity of the parasites to each tested fractions at concentration of 500, 1000, 2000, and 5000µg/ml was determined under microscopically at 37^{o}C after 24 and 48 hours of incubation. The incubation was carried out under a bell jar system with a lighted candle that ensured the condition being atmospherically inert (about 5% O_2, 2% CO_2 and 93% nitrogen gas) as demonstrated by Mukhtar *et al.,* (2006).

3.2.3.7 Determination of Activity of Parasitemia Clearance of the extract.

At the end of each incubation period, a drop of a thoroughly mixed aliquot of the culture medium was smeared on microscopic slides and stained by Leishman's staining techniques. The mean number of erythrocytes appearing as blue discoid cells containing life rings of the parasite (that appeared red pink) was estimated and the average percentage elimination by the samples was determined. The activity of the tested samples was calculated as the percentage elimination of the parasites after each incubation period, using the formula below;

$$\% = \frac{\mathrm{N}}{\mathrm{Nx}} \times 100$$

Where, % = Percentage inhibition of the parasites

N = Total number of cleared RBC

Nx = Total number of parasitized` RBC

3.3. Characterization of the most active Extract Fraction (chloroform)

3.3.1 Analysis

The Gas chromatography Mass spectrometry (GCMS) analysis of chloroform partitioned extract of *Senna ccidentalis* was carried out at Multi user laboratory , Zaria-Nigeria using GC-MS,(Model, QP 2010 PLUS, Shimadzu, Japan). Equipped with a VF-5MS fused silica capillary column of 30m length, 0.25mm diameter and 0.25µm film thickness. The column oventemperature was programmed from 80°^{C} to280°^{C} for 2 min-1. Ionization of the sample components was performed in electron impactmode (EI, 70 eV). The temperature of the injector was fixed to 250°C and one of the detector to 200°C. Helium (99.9995% purity) was the carrier gas fixed with a flow rate of 1.5 mlmin-1. The mass range from 40-1000 m/z was scanned at a rate of 3.0 scans/s. One micro liter (1.0µl) of the extract samples was injected with a Hamilton syringe to the GC-MS manually for total ion chromatographic analysis (TIC) analysis in split injection technique. Total running time of GC MS is 45min.

The relative percentage of the each extract constituents was expressed as percentage with peak area normalization.

3.3.2 Identification of component

The identity of the bioactive compounds in the chloroform fraction of *Senna occidentalis*was carried out by GC-MS based on the comparison of their retention indices and mass spectra fragmentation patterns with those stored on the computer library (i.e. The Spectrum of the unknown component was compared with the spectrum of known components stored in the NIST

Library) and the Interpretation of mass spectrum was conducted using data base of National Institute of Standards Technology (NIST) and Fatty AcidMethyl Esters Library version 1.0 (FAME library) sources were for matching the identified components in the extract. The molecular weight, molecular formula and the number of hits used to identify the name of the compound from NIST Libraries were recorded.

3.4 Toxicological Studies of *Senna occidentalis* Leaves Extract

A total of 20 male albino rats were used and they were divided into four groups of five rats each. The extract was administered daily orally to the rats in groups II to IV at a dose of 100mg/kg body weight (low), 200mg/kg body weight (normal), and 300mg/kg body weight (high). Group I rats serve as control. All the rats were sacrificed after 28days of extract administration, blood samples was collected and centrifuged for analysis of liver and kidney functions indeces.

3.4.0 ANALYSIS OF LIVER FUNCTION MARKERS

3.4.1 Aspartate Aminotransferase Assay (AST) method described by Reitman and Frankel (1957) was used

Principle

Aspartate aminotransferase (AST), previously known as glutamate oxaloacetate transaminase (GOT) catalyses the transamination reaction between aspartate and α-ketoglutarate to produce oxaloacetate, the oxaloacetate form react with 2,4-dinitrophenyl hydrazine to produce hydrazone.

α-ketoglutarate + L-aspartate $\xrightarrow{\text{AST}}$ Glutamate + oxaloacetate

AST is measured by monitoring the concentration of hydrazone formed with 2,4-dinitrophenyl hydrazine colorimetrically at 546 nm.

Procedure,

Two test tubes were labeled as sample and sample blank. Reagent R_1 (phosphatebuffer, L-aspartate,oxoglutarate) (0.5ml) was added into the both tubes. Serum ($0.1cm^3$) was pipetted into the sample tube. They were then mixed and incubated for exactly 30min at 37^0C, Reagent R2 (2,4 dinitrophenylhydrazine) (0.5ml) was pipetted into both tubes followed by 0.1ml of distilled water into sample blank, mixed and allowed to stand for 20mins at 25^oC. Sodium hydroxide (0.5ml) was pipetted into both tubes, mixed and the absorbance of sample against the sample blank after 5mins was read at 546nm. The activity of AST was obtained by comparing the absorbance with the activity value given from the Randox kit manual.

3.4.2 Serum Alanine Aminotransferases Assay (ALT)

Method described by Reitman and Frankel, (1957) was used.

Principle

ALT catalyses the transfer of the amino group from alanine to α-ketoglutarate forming pyruvate and glutamate. The pyruvate reacts with 2, 4-dinitrophenyl hydrazine (DNPH) to form 2, 4-dinitrophenyl hydrazone which in an alkaline medium gives a red-brown colour. The absorbance of the colour produced is measured by colourimeter using a green filter at 520 wavelength.

α-ketoglutarate + L-alanine $\xrightarrow{ALT}$ Glutamate + pyruvate

ALT is measured by monitoring the concentration of pyruvate hydrazone formed with 2,4-dinitrophenyl hydrazine colorimetrically at 546 nm.

Procedure

Two test tubes were lebelled as sample and sample blank, solution R_1(phosphate buffer, 100mml/L , PH 7.4; L- alanine,oxoglutarate) ($0.5cm^3$) was added into both tubes. Serum ($0.1cm^3$) was pipetted into the sample tubes. They were then mixed and incubated for 30min at

37^0C. Solution R_2(2,4 dinitro phenylhydrazine) (0.5cm^3) was added into both tubes followed by 0.1cm^3 of distilled water into the sample blank. They were then mixed and allowed to stand for exactly 20min. at 25^0C. Sodium hydroxide (5 cm^3) was then pipetted into the both tubes mixed and the absorbance of the sample against the sample blank was read and recorded after 5 minutes at 546nm.The activity of ALT was obtained by comparing the absorbance with the activity value given from Randox kit manual.

3.4.3 Serum Alkaline Phosphatase (ALP)

Method described by Rec, (1972) was used in serumALP Determination.

Principle

ALP catalyzes the hydrolysis of P-nitrophenylphosphate to give p-nitrophenol and inorganic phosphate. In the phenol is condensed with 4-amino anti pyrene and then oxidized with alkaline ferricyanide to give a coloured complex which is measure it 456 nm.

P-nitrophenyl phosphate + H_2O $\xrightarrow{ALP}$ Phosphate + P- nitrophenol

Procedure

1cm^3 of reagent was put in a cuvette, 0.50cm^3 of serum was then added. The solution was mixed and the absorbance ($A_{initial}$) was read and recorded immediately and timing was started simultaneously. The absorbance was read after first, second and third minutes against air blank at 405nm.

Activity

The activity of ALP was obtained by using the formula

U/L = 2760 x ΔA 405nm/min

Where ΔA: Difference in absorbance

3.4.4 Determination of serum Total Protein

Method described by Tietz (1995) was used.

Principle

Cu^{2+} reacts in alkaline solution with the peptide linkages of protein to form a violet coloured complex. The intensity of colour is proportional to the protein concentration. A structure containing at least two peptides is required to form the complex.

Procedure

Into three test tube label test standard and blank, 5.0ml of biuret reagent was added followed by 0.1ml of sample, standard and blank into their respective tube. The entire content was mixed and allowed to stand for 30 minutes, absorbance were read and recorded at 550nm and blank was used to zero colorimeter.

Calculation

$$Total\ protein\ (g/dl) = \frac{Absorbance\ of\ test}{Absorbance\ of\ std} \times [std]$$

3.4.5 Serum Albumin Determination described

Method described by Doumas, *etal*, (1971) was used.

Principle

The method is based on the ability of albumin to bind various substances. The most widely used dye is bromocresol green because it is less subject to interferences. Albumin binds with bromocresol green at pH 4.2 to give a related colour.

Procedure

Into three test tubes label test standard and blank, 2.0ml of bromocresol green was pipettes followed by 0.02ml of serum, standard albumin and distilled water to their respective test tube.

The content was mixed and allowed to stand for 10 minutes. Blank reagent was used to zero the colorimeter which was set at 630nm and absorbance were read and recorded accordingly.

Calculation

$$[\text{Albumin}]\ (\text{g/dl}) = \frac{Absorbance\ of\ test}{Absorbance\ of\ std} \times [std]$$

3.4.6 Determination of Bilirubin

Method described by Tietz, (1990) was used.

Principle

Direct bilirubin (conjugated) bilirubin reacts with diazotized sulphanilic acid in alkaline medium to form a blue coloured complex. Total bilirubin is determined in the presence of caffeine, which releases albumin bound bilirubin, by the reaction with diazotized sulphanilic acid.

Procedure

a. Total Bilirubin

Into test tubes labelled sample and sample blank, 200µl of reagent (sulphanilic acid mmol/L) was added to each test tube followed by 50µl of reagent (sodium nitrites 50 mmol/L) into sample only and then 1000µl and 200µl of reagent (Caffeine 100mmol/L) and sample was then added to each test tube. The content was mixed and allowed to stand for 10mins at 20^0C. (Soduim oxide) (1000µl) was added to each tube and incubated for further 30mins. The absorbance of sample and sample blank were read at 578nm.

Calculation

Total bilirubin conc. (mg/dl) = $10.8 \times A_{TB}$

b. Direct bilirubin

Into test tubes labelled sample and sample blank, 200µl of reagent 1 was added to each test tube followed by 50µl of reagent 2 into sample only and then 2000µl of 0.9% NaCl and 200µl sample

was added to each test tube. The content was mixed and allowed to stand for 10mins at 20^0C. The absorbance of sample and sample blank were read at 546nm.

Calculation

Direct bilirubin conc. (mg/dl) = $14.4 \times T_{AB}$

3.5 ANALYSIS OF KIDNEY FUNCTION INDICES

3.5.1 Determination of Serum Creatinine

Method described by Bartels and Bohmer (1972) wasused.

Principle

Creatinine in alkaline solution reacts with picric acid to form a coloured complex. The amount of the colour complex is directly proportional to the creatinine concentration.

Procedure

Three test tubes were labelled as blank, standard and sample. Working reagent (Alkaline buffer and picric acid 40mM) ($2cm^3$) was pipetted to all test tubes. Sample and calibrator ($0.2cm^3$) each was added to the respective test tubes and $0.2cm^3$ of distilled water to the blank. The mixtures were shaken and allowed to stand at room temperature for 30mins. The absorbance was read at 490nm against that of blank

Calculation

$$\text{Creatinine (g/dl)} = \frac{Absorbance\ of\ test}{Absorbance\ of\ std} \times [Conc.of\ Std\ \ (mmol/L)$$

3.5.2 Determination of Serum Urea

Method described by Weatherburn, (1967) was used.

Principle

Urea in serum is hydrolyzed to ammonia in the presence of urease. The ammonia is then measured photometrically by Berthelot's reaction.

$Urea + H_2O \xrightarrow{urease} NH_3 + CO_2$

NH_3 + Hypochloride + phenol → Indophenol (Blue compound)

Procedure

Three test tubes were labeled as blank, standard and sample. Distilled water, standard and sample (10µl) each were put into the three test tubes respectively. (Ureaseoxide) (100µl) was then added into each tube. They are mixed immediately and incubated at 37^{o}C for 15mins. The absorbance of the sample (A_{sample}) and standard ($A_{standard}$) were measured against the reagent blank at 546nm.

Calculation

$$\text{Urea concentration (mmol/L)} = \frac{Absorbance\ of\ test}{Absorbance\ of\ std} \times (concentration\ of\ Std)$$

3.5.3 Determination of Sodium

Method described by Maruna (1958) was used.

Principle

The method is based on modifications of those first described by Maruna in which sodium is precipitated as the triple salt, sodium magnesium uranyl acetate, with the excess uranium then being reacted with ferrocyanide, producing a chromophore whose absorbance varies inversely as the concentration of sodium in the test specimen.

Procedure

Three test tubes were labelled as test, standard and blank. $Mgcl_3(1.0cm^3)$ was pipetted to all tubes followed by 50µl of sample, standard and distilled water into test, standard and blank respectively. All tubes were shaken and mixed continuously for 3mins and centrifuged at 1500G for 10mins. To test the supernatant obtained, $1.0cm^3$ of acid reagent was pipetted into test and standard followed by 50µl of supernatant and colour reagent respectively. The tubes were then mixed while spectrophotometer was zero with distilled water at 550nm and the absorbance of all tubes was recorded.

Calculation

$$\text{Concentration of sodium (mmol/L)} = \frac{Absorbance\ of\ test}{Absorbance\ of\ std} \times (\text{Conc. of Std})$$

3.5.4 Determination of serum Potassium

Method described by Terri, *etal*.(1958) was used.

Principle

The amount of potassium is determined by using sodium tetraphenylboron in a specifically prepared mixture to produce a colloidal suspension. The turbidity of which is proportional to the potassium concentration.

Procedure

Three test tubes were labelled as test, standard and blank. Soduim bromide ($1.0cm^3$) was pipetted to all tubes followed by 10µl of sample and standard to respective tubes and allowed to stand at room temperature for 3mins. Reagent blank was used to zero the spectrophotometer at 500nm then the absorbance of all tubes was read and recorded.

Calculation

$$\text{Concentration of potassium (mmol/L)} = \frac{Absorbance\ of\ test}{Absorbance\ of\ std} \times \text{(Absorbance of Std)}$$

3.5.5 Determination of Serum Chloride

Method described by Tietz (1976) was used.

Principle

Chloride ions form a soluble, non-ionized compound, with mercuric ions and will displace thiocyanate ions which react with ferric ions to form a color complex that absorbs light at 480nm. The intensity of the color produced is directly proportional to the chloride concentration.

$Hg(SCN)_2 + 2Cl^- \rightarrow HgCl_2 + 2SCN^-$

$3SCN^- + Fe^{3+} \rightarrow 4Fe(SCN)_3$ Red complex

Procedure

Three test tubes were labelled as test, standard and blank. Chloride reagent (mercury) ($1.5cm^3$) was pipette to all tubes followed by 10µl of samples, standard and distilled water to respective tubes and allowed to stand at room temperature for 5 minutes. Reagent blank was used to zero the spectrophotometer at 480nm then the absorbance of all tubes was read and recorded.

Calculation

$$\text{Concentration of chloride (mmol/L)} = \frac{Absorbance\ of\ test}{Absorbance\ of\ std} \times \text{(Absorbance of Std)}$$

3.5.6 Determination of Serum Bicarbonate

Method described by Tietz *et al .,(*1986) was used.

Principle

Phosphoenol pyruvate carboxylase (PEPC) catalyzes the reaction between phosphoenol pyruvate and carbon dioxide (bicarbonate) to form oxalacetate and phosphate ion. Oxalacetate is reduced

to malate with simultaneous oxidation of an equimolar amount of reduced nicotinamide adenine dinucleotide (NADH) to NAD, the reaction is catalyzed by malate dehydrogenase (MDH). This results in a decrease in absorbance at 340nm that is directly proportional to CO_2 concentration in the sample.

Phosphoenol pyruvate + HCO_3^- $\xrightarrow{PEPC}$ Oxalate + H_2PO_4

Oxalate + NADH $\xrightarrow{MDH}$ Malate + NAD

Procedure

Three test tubes were labelled as sample, standard and blank. Carbon dioxide reagent (1.0ml) was pipetted into all tubes and incubate for 3mins at 37^0C. Followed by 5.0µL of distilled water, standard and blank into tubes labelled blank, standard and test respectively. All tubes were then mixed and incubated for 5mins and the absorbance of all tubes was read at 340nm.

Calculation

$$\text{CO2 content of sample (mmol/L)} = \frac{Absorbance\ of\ test}{Absorbance\ of\ std} \times (\text{Conc. of Std})$$

3.6 Statistical Analysis

Results were expressed as mean ± standard deviation. Statistical differences between groups were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's test after investigating the data for normality using Shapiro-Wilk test and for variances homogeneity to be sure that the data are normally distributed and variances would be homogenous using GraphPad Instat3 Software version 3.05 .Differences at $P < 0.05$ were considered to be significant.

CHAPTER FOUR
RESULTS AND DISCUSSION

4.1 RESULTS

The physical properties of the aqueous methanolic extract of *Senna occidentalis*(table 4.1) yielded 12.5 % after 100g of the powdered leaves. The extract was observed to be dark brown in colour with a sticky exture.

Table 4.1: Physical properties of the extract of *Senna occidentalis*leaves

Property	
Weight of plant(g)	100
Weight of extract(g)	12.5
Percentage yield(%)	12.5
Colour of extract	Dark brown
Texture of extract	Sticky

Antiplasmodial properties of the aqueous-methanol of *Senna occidentalis*leaf extract, including Positive and negative control on were presented in the Table 4.2Theaqueous-methanol of *Senna occidentalis*leaf extract gave the highest parasite clearance of 85.41.0% at 5000µg/ml,while least activity was found in the concentration of 500 µg/ml of the same extract. Treatment with the standard drug ACT resulted in exponential decrease in parasite count with giving the highest curative activity of 97.05%. However, the negative control showed no decrease in parasite count.

Table 4.2:Anti-Plasmodial Properties of Aqueous-methanol Leaf Extract of *Senna occidentalis*.

Extracts	**Conc.(µg/ml)**	**Initial count**	**Average no. during incubation**			**Average no. After Incubation**	**%elimination**	**IC_{50}** µg/ml
			24 hrs	**48 hrs**	**72 hrs**			
Control	ACT	34	1	0	0	1.00	97.05480	
	Negative	34	34	34	34	34	0	
	5000		21	21	20	20.66±0.12	30.48	
	2000		26	27	27	26.70±0.16	21.47	
	1000		30	29	29	40.57±0.14	18.86	
	500		30	30	31	31.03±0.34	7.25	
Aqueous-methanol								
	5000		6	4	4	4.66±0.13	85.41	370
	200		13	15	15	14.33±0.10	84.00	
	1000		22	20	19	20.80±0.20	38.8	
	500	34	28	27	27	27.30±0.16	19.90	

`

Antiplasmodial properties of *Senna occidentalis*leaf extract on *Plasmodiumfalciparum* were detected in all the partitioned fractions of the extract, namely chloroform Ethylacetate, and N-hexane partitioned fractions .Chloroform partitioned fractions gave the highest parasite clearance of 94.64.0% and 93.75 at 5000µg/ml and 2000µg/ml respectively. Least activity of 16.66% at the concentration of 500µg/ml was found in N-hexane partitioned fraction. ACT was used as a standard drug though gave the highest parasite clearance of 97.05%. However, the negative control showed no decrease in parasite.

Table4.3: Anti-plasmodial Properties of Solvents Fractions from Crude *Senna occidentalis*

Extracts	Conc. (µg/ml)	Initial counts	Average no. during Incubation			Average no. After incubation	% Elimination	IC_{50}µg/ml
			24hrs	48hrs	72hrs			
Ethylacetate	5000	56	16	15	15	15.33±0.167	2.6	250
	2000	20	18	18	18.60 ±0.506	7		
	1000	28	28	28	28.30 ±0.874	9.3		
	500	35	35	35	35.00 ±1.543	7.5		
Chloroform	5000	56	3	0	0	3.00 ±0.679	4.64	430
	2000	4	4	3	3.50 ±1.123	3.75		
	1000	8	6	6	6.66 ±0.788	8.2		
	500	9	9	8	8.30±0.458	5.17		
N-hexane	5000	56	26	23	22	23.68 ±0.115	7.70	280
	2000	30	28	26	28.10 ±0.894	9.47		
	1000	32	29	27	29.33 ±0.444	7.20		
	500	49	47	44	46.66 ±0.636	.66		

4.1.2 Impact of*Senna occidentalis*Aqueous methanol leaves extract on liver function indices

The effect of administration of varying doses of the crude extract on liver function indices (AST, ALT, ALP, DB, TB, TP and ALB) is shown in Table 4.4 The result showed a significant ($p<0.01$) decrease in level of AST,ALP,ALT,TP and ALB in a dose dependent pattern compared to normal control. The result showed no significant effects on ALT and AST at alltreatment doses. This suggests that administration of aqueous methanol leaf extract of *Senna occidentalis*has no hepatotoxic effects in rats. An increase in mean serum of direct bilirubin and total bilirubin was also observed in the administered groups when compared to normal control though not significant. In the present study also, the extract did not cause a significant change in total protein and albumin.

Table 4.4 Liver function indices (AST, ALT, ALP, DB, TB, TP and ALB) of rats administered with the aqueous methanolic crude extract for six weeks

Group	ALT(IU/L)	AST(IU/L)	ALP(IU/L)	DB(mg/dl)	TB(mg/dl)	TP(g/dl)	ALB(g/dl)
Control	29.43±2.90 [a]	23.897±2.02 [a,b,c]	56.96±1.85[a,b,c]	0.34±0.06[a,b]	1.65±0.16[a]	7.01± 1.11	5.97±0.16[a]
100mg/kg /bdw	26.01±1.72[b]	19.26±0.08[a]	44.91±.3.04[a,]	0.47±0.03	1.31±0.34	7.16±1.24	5.70±0.82
200mg/kg /bdw	20.65±16.73	15.50±3.00[b]	44.46.±4.04[b]	0.98±0.03[a]	2.05±0.38	7.86±1.32	5.46±0.33
300mg/kg /bdw	20.05±2.63	14.7±0.8[c]	36.23±2.05[c]	1.4±0.28[b]	2.77±0.10[a]	5.63±2.03	5.19±0.95[a]

Values are presented as Mean ± standard deviation, (n=5). Value with the same superscripts in a column are significantly different compared to each other ($P<0.05$). ALT, alanine transaminase,AST, Aspartate transaminase,ALP, Alkaline phosphatase,DB, Direct bilirubin,TB, Total bilirubin,ALB, Albumin.

4.1.2 Impact of*Senna occidentalis*Aqueous methanol leaves extract on kidney functionindices.

Table 4.5 shows the serum concentrations of urea, creatinine and electrolytes (Na^+, Cl^-, K^+, HCO_3^-) of rats at the end of administration of the extract. A significant increase ($p<0.05$) was observed in mean serum urea,soduim creatinine and potassuim of the extract administered groups while a significant decrease ($p<0.05$) in mean serum chloride and HCO_3^- was observed in extract administered groups compared to normal control.

Table 4.5: Kidney function indices of rats administered with administered with the aqueous methanolic crude extract for six weeks

Group	Urea(mmol/L)	Crea(mmol/L)	Na^+(mmol/L)	K^+(mmol/L)	Cl^-(mmol/L)	HCO_3^- (mmol/L)
Control	$20.85\pm0.82^{a,b,c}$	$20.83\pm4.31^{a,b,c}$	$93.47\pm0.18^{a,b}$	3.59 ± 0.54	96.59 ± 0.54	23.89 ± 3.45
100mg/kg /bdw	44.89 ± 4.18	21.2 ± 1.39^{a}	$107.92\pm2.86^{a,}$	4.30 ± 0.58	90.18 ± 2.35	21.78 ± 4.84
200mg/kg /bdw	45.12 ± 2.57	25.68 ± 1.42^{b}	106.83 ± 5.62^{b}	5.45 ± 1.21^{a}	85.49 ± 2.32	$21.22\pm4.22.$
300mg/kg /bdw	38.10 ± 5.71^{a}	25.36 ± 0.25^{c}	116 ± 5.62^{c}	7.05 ± 0.56^{b}	87.88 ± 2.38	18.68 ± 1.90

Values are presented as Mean ± standard deviation, (n=5). Value with the same superscripts in a column are significantly different compared to each other ($P<0.05$). crea, creatinine ,Na sodium,K Pottassuim,Cl, chloride, HCO_3^-, bicarbonate.

4.3 Identification of chemical components.

Figure 4.2 shows the GCMS chromatogram of most active chloroform fraction. The spectra shows the presence of Fucalyptol,Octadecane isocyanato,2-hexanolyfuran 1H-imidazole ,1-ethyl propen Isopropyl imidazole, Oxabicyclo[6.1.0]nonane , 2-pentadecanal 2-heptadecanalPentadecanoic acid acid,1,5-DecadiyneCyclopentane, butyl- 1-Chloroundecane Hexadecanoic acid, methyl ester 2-Pentadecanol 1-Hexacosanol 6-Octadecenoic acid, methyl ester, (Z)- Heptadecanoicacid, 16-methyl, methyl ester n-Hexadecanoic acid Oleic 2-Hydroxymethylcyclopentanol (cis)12-Oleanen-3-yl acetate (3.alpha) (beta amyrin acetate)Oxalic acid, di(cyclohexylmethyl) ester Chloroacetic acid, 4-pentadecyl ester Sesquirosefuran , Lupeol Urs-12-en-3-ol,acetate(3 beta) (alpha amyrin), Lup-20(29)-en-3-ol acetate 12-Oleanen-3-yl acetate and 48.66Benzoic acid, 3,5-dicyclohexyl- were detected in the chloroform extracts.

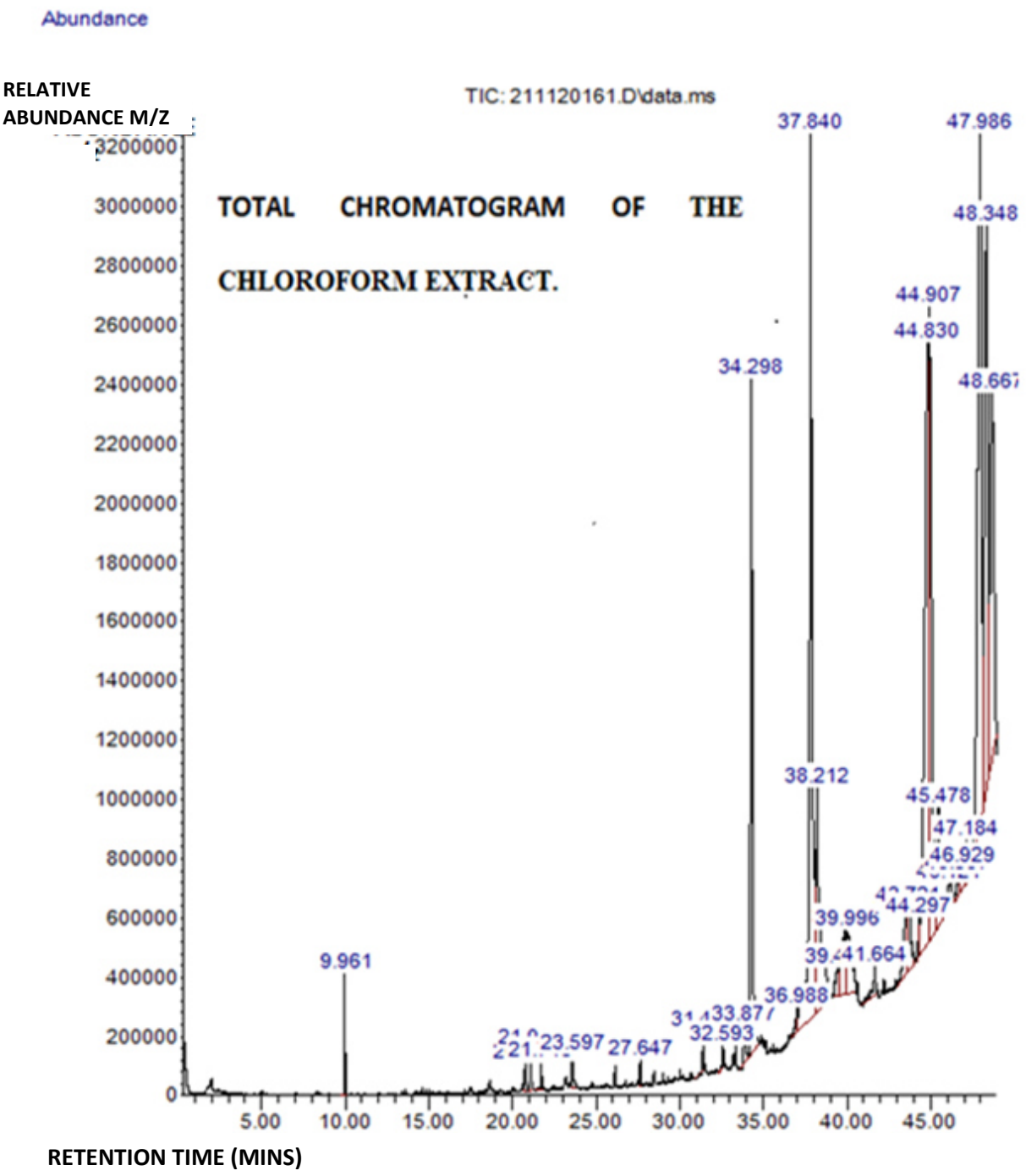


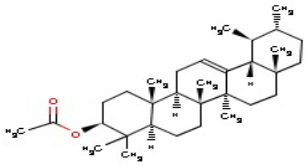
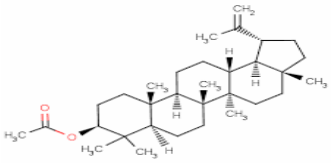
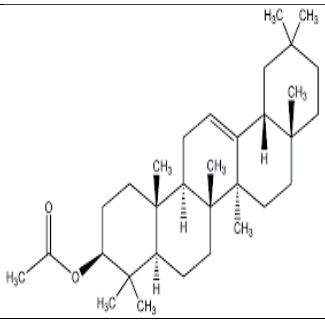
Figure 4.2: GC-MS chromatogram of chloroform extract.

4.4GC-MS characterization of chloroform partitioned extract of *senna occidentalis*

S/N	RT	Name of Compound	MF	MW(g/mol)	Area %
1.	9.961	Fucalyptol	$C_{10}H_{18}O$	154.249	0.51
2.	20.76	Octadecane isocyanato	$C_{19}H_{37}NO$	295.511	0.26
3.	21.08	2-hexanolyfurane	$C_{15}H_{32}$	212.421	0.26
4.	21.71	1H-imidazole ,1-ethyl propen	$C_{14}H_{30}$	198.394	0.20
5.	23.59	Isopropyl imidazole	$C_{12}H_{12}$	156.228	0.29
6.	27.64	Oxabicyclo[6.1.0]nonane	$C_{16}H_{34}$	226.448	0.14
7.	31.42	2-pentadecanal	$C_{18}H_{30}$	246.438	0.22
8.	33.87	2-heptadecanal	$C_{18}H_{30}$	246.438	0.13
9.	34.29	Pentadecanoic acid acid	C_5H_{10}	246.438	0.40
10.	36.98	1,5-Decadiyne	$C_{10}H_{14}$	134.222	6.71
11.	37.84	Cyclopentane, butyl-	C_9H_{18}	126.243	0.26
12.	38.21	1-Chloroundecane	$C_{11}H_{23}Cl$	190.755	14.10
13.	39.48	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	228.42	4.98
14.	39.82	2-Pentadecanol	$C_{15}H_{32}O$	426.729	2.76
15.	39.99	1-Hexacosanol	$C_{26}H_{54}O$	382.717	3.53
16.	41.61	6-Octadecenoic acid, methyl ester, (Z)-	$C_{19}H_{362}$	296.495	9.93
17.	41.66	Heptadecanoic acid, 16-methyl, methyl ester	$C_{19}H_{38}O_2$	298.511	6.65
18.	43.55	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.43	3.25
19.	43.72	Oleic Acid	$C_{18}H_{34}O_2$	282.468	3.54

S/N	RT	Name of Compound	MF	MW(g/mol)	Area %
20	44.83	2-Hydroxymethylcyclopentanol (cis)	$C_6H_{12}O_2$	116.158	44.29
21	44.90	Urs-12-en-3-ol, acetate, (3.beta.)	$C_{32}H_{50}O_4$	456.7	44.88
22	45.49	12-Oleanen-3-yl acetate, (3.alpha) (beta amyrin acetate)	$C_{32}H_{50}O_4$	456.7	44.83
23	45.47	Oxalic acid, di(cyclohexylmethyl) ester	$C_{12}H_{20}O_4$	44.90	3.07
24	46.12	Chloroacetic acid, 4-pentadecyl ester	$C_{10}H_{17}ClO_2$	46.12	0.82
25	46.59	Sesquirosefuran	$C_{15}H_{22}O$	218.33	0.34
26	47.18	Lupeol	$C_{30}H_{50}O$	426.72	0.41
27	32.90	i-Propyl 9-octadecenoate	$C_{21}H_{40}O_2$	112.8	0.34
28	47.98	Urs-12-en-3-ol, acetate, (3.beta)	$C_{32}H_{50}O_3$	468.8	16.40
29	48.34	Lup-20(29)-en-3-ol, acetate	$C_{32}H_{52}O2$	468.75	10.71
30	48.66	Benzoic acid, 3,5-dicyclohexyl-4-	$C_{20}H_{28}O_3$	316.4	8.11

Table 4.7: Summary of some possible compounds identified in chloroform fraction extracts by GCMS techniques with possible antimalrial activity.

COMPOUND	PEAK#	R.TIME	HEIGHT%	MOLECULAR FORMULA	DERIVED STRUCTURE
Urs-12-en-3-ol,acetate(3 beta) (alpha amyrin).	21	44.90	2.51	$C_{32}H_{52}O_2$	
Lup-20(29)-en-3-ol, acetate LUPENYL ACETATE	29	48.3	9.21	$C_{32}H_{52}O_3$	
12-Oleanen-3-yl acetate, (3.alpha) (beta amyrin acetate)	22	47.98	9.21	$C_{32}H_{53}O_2$	

4.5 Discussion

Aqueous Methanol was used in the extraction of*Senna occidentalis*leaf due to the fact that methanol can extract both polar and non-polar compounds to some extent. Yield of 12.5% signifies that methanol was good in the extraction of the phytochemicals from the dry leaves of *Senna occidentalis*. This is in agreement with that of Alhassan *et al*2017 on the effect of aqueous root extract of *Senna occidentalis* on acetaminophen inducced hepatorenal toxicity rat model.

The search for drugs and dietary supplement from plants to treat and or manage malaria has accelerated in recent years which nessiacitate this research. *Senna occidentalis*isone of the plant with long history of traditional use in treatment of malaria, hepatitis, wound healing etc(Alhassan *et al*., 2017).The antiplasmodial properties of fractions of *Senna occidentalis*leaf (Table 4.2 and 4.3)was observed during three days treatment and parasitaemia counts on the interval of 24 hrs was recorded. Treatment with the crude Aqueous methanol extracts and the partitioned fractions of leaf extract resulted in exponential decrease in parasite count throughout the study period , with chloroform partitioned fraction showing the highest clearance activity, followed by Ethylacetate and least was N-hexane. Artismenia combine theraphy gave the highest clearance activity by clearing almost all the parasite completely by the 3rd day of treatment. At concentrations of 5000, 2000 and 1000 and 500 µg/ml, chloroform partitioned extracts produced the highest curative activity. From literature, an extract is regarded as highly active if the percentage clearance is greater than 50% (Ramazani *et al*, 2010). It can be seen that the average number of parasites increases as the `concentration of the extract decreases. This indicates that higher concentrations of the extracts were found to be more effective on the parasites.

The antimalarial activities of *Senna occidentalis* may be linked Alterations of the erythrocyteshape were also observed with lupeol, and other triterpenoids (Urs-12-en-3-ol, acetate (3 beta) and 12-Oleanen-3-yl acetate, (3 alpha) (beta amyrin acetate) (alpha amyrin).The majorantiplasmodial constituent isolated from the plant *R. ilicifolia*.It has previously been described that lupeol exhibits inhibitoryactivity on *P. falciparum* growth in vitro but lacks in vivo activityin mice infected with *P. berghei* (Alves *et al.,* 1997). However, no informationabout the mechanism of the in vitro activity was reported.It is now shown that lupeol causes membrane shapechanges of erythrocytes toward stomatocytic forms observableat concentrations below its IC50 value. The compound inducedendovesiculation, which is characteristic of stomatocytogeniccompounds (Hgersrend and Singer 1974). There is a good correlation between the lupeol concentration at which morphological changes in erythrocytesoccur and the observed IC50 of many compounds. This strongly suggests that the in vitro antiplasmodial mactivity of lupeol is indirect, being due to membranemodification of the host cell.The structure of lupeol is reminiscent of that of cholesterol,and the compound is expected to be able to enter the cellularmembranes. Due to the presence of a single hydroxy group anda large, apolar skeleton, lupeol acts as an amphiphile.

According to the bilayer hypothesis (Shertz and Singer, 1974), stomatocytes aregenerally formed when a lipophilic compound is incorporatedinto and expands the inner layer of the lipid membrane. Suchchanges appear to be more prohibiting with respect to parasitegrowth than incorporation of an amphiphile into the outerlayer, as in case of echinocytogenic compounds. It is demonstrated that the inhibition of parasite growth does not require the presence of lupeol in the growth medium, sinceerythrocytes preincubated with lupeol proved to be unsuitablefor parasite cultivation this strongly suggests the permanentincorporation of lupeol into the lipid

bilayer. Thepresence of an excess of extracellular merozoites in a cultureemploying erythrocytes pretreated with lupeol suggests thatthe invasion of the erythrocytes has been impaired also.In an inverse experiment relative to that described above, aparasite culture was treated with lupeol and subcultured withuntreated erythrocytes. In an experiment, the time ofpreincubation had to be limited to 3 to 6 h; otherwise, theparasites would die. In spite of the pretreatment with lupeol,the parasites grew normally in untreated cells after removal of lupeol. Thus, the ability of the parasites to invade andgrow in fresh erythrocytes was not impaired by the initial exposureto lupeol.Previous studies have demonstrated that alterations ofthe erythrocyte membrane such as cross-linking of spectrin,changes in deformability, spherocytosis, and modification ofthe cytoskeletal proteins have inhibitory effects on invasion (Dluzewski *et, al.,* 1983). No studies of incorporation oflupeol into erythrocytes and its effect on parasite proliferationhave been reported prior to this work. A recent report ofVidaya et al. (Vidaya *et al.,* 2000) showing that erythrocytes of rats fed withlupeol exhibit altered osmotic fragility is compatible with thisfindings.

Although the exact mechanism by which stomatocytosismakes the erythrocytes unfavorable for *P. falciparum* invasionand growth has yet to be elucidated, the present findings are ofinterest for drug discovery programs based on natural products.Lupeol and other pentacyclic triterpenes and sterols withrelated structures are very common constituents of plants andare thus frequently encountered in plant extracts used forscreening. Many synthetic drug candidates may also act as stomatocytogenic amphiphiles. The membrane alterations thatinhibit parasite growth take place long before they can bedetected by routine examination by optical microscopy and thus care has to be exercised when *P. falciparum* in vitrogrowth inhibition results are interpreted.

4.5.1 Impact of *Senna occidentalis* aqueous methanol leaf extract on kidey and liverfunction

Liver is an important body organ and actively involved in different metabolic functions (Meyer and Kulkarni, 2001). Hepatic damage caused by chemicals or infectious agents is associated with distortion of these metabolic functions and may lead to progressive liver fibrosis and ultimately cirrhosis and liver failure. Liver damage is a serious disease characterized by disturbances in the normal functions of the liver. It is clinically diagnosed by determining the serum concentration of liver enzymes (ALT, AST and ALP), bilirubin, total protein and albumin. ALT is an enzyme normally present in liver and heart cells. When the liver or heart is damaged, ALT in blood will increased, and thus indicates liver or heart injury. During hepatocellular injury, enzymes which are normally located in the cytosol are released into the blood flow. Their qualification in plasma is useful biomarkers of the extent and type of hepatocellular damage (Pari *et al.,* 2004) ALT and AST are used to assess the hepatocellular integrity of liver tissue. ALT is more predominantly found in liver while AST is normally found in equal amounts in the liver, heart, muscle, kidney and brain. Therefore, ALT is more liver-specific than AST (Anderson *et al.,* 2003). ALP is also a non-plasma specific enzyme involved in the hydrolysis of a variety of phosphate esters at alkaline pH. These enzymes were reported to reach higher than normal level in the blood in events of impaired liver function (Price and Stevens, 2003). Thus, they are used as serum markers of hepatic damage.

Liver enzymes, aspartate and alanine aminotransferases (AST and ALT) are involved in amino acid metabolism. Large amounts of AST are present in the liver, kidney, cardiac muscle, and skeletal muscle (Hassan *et al.,* 2007). Serum ALT and AST are always found to increase in liver cell damage and the greater the degree of the liver damage the higher the activities of both enzymes (Cheerburgh,2005). The result (Table 4.4.) showed no significant effects on ALT and

AST at all treatment doses.Bilirubin, a metabolic breakdown product of haem derived from senescent red blood cells (Rosalki and Mcintyre, 2010). Bilirubin is a useful index of the excretory function of the liver, in addition to its being auseful tool in assessment of haemolytic anaemia. Raised serum levels of direct and total bilirubin is an indication of an impaired hepatic excretion (whitly *et al.,* 1989).

The assay of total protein alone may not portray the true picture of the metabolic state of an individual since the concentration of individual proteins do not rise or fall in parallel with one another. Albumin is the most abundant of the plasma proteins with the physiological role of maintenance of osmotic pressure, transportation of endogenous and exogenous substance and serving as protein reserve. The ability of the liver to synthesise albumin is diminished if the ynthetic function of the organ is affected. Rapid reduction in serum albumin is therefore apparent in hepatitis and liver cirrhosis. In the present study, the extract did not cause a significant reduction in total protein and albumin. This suggests administration of aqueous methanol leaf extract of *Senna occidentalis*as no hepatotoxic effects in rats.

Kidneys are the major organs in metabolizing toxic campound besides liver. It receives about 1200ml of blood per minute (Tortora *et al.,* 2006) containing a lot of chemical compounds. Therefore damage to the kidneys can be determined by measuring the level of urea, electrolyte and creatinine in blood as an indicator of kidney damage. Urea is a byproduct from protein breakdown. About 90% of urea produced is excreted through the kidney. Meanwhile, the creatinine is a waste product from a muscle creatinine, which is used during muscle contraction. Creatinine is commonly measured as an index of glomerular function, it is excreted exclusively through the kidney. Therefore, damage to the kidney will make the kidney inefficient to excrete both urea and creatinine and causes their accumulation in the blood. Therefore, the high level of

blood urea and creatinine will indicate kidney damage also Electrolytes are substances that become ions in solution and acquire the capacity to conduct electricity. The balance of the electrolytes in human bodies is essential for normal function of cells and organs. An electrolyte is a substance that Common electrolytes include sodium, potassium, chloride, and bicarbonate. The functions and normal range values for these electrolytes are important, and if an electrolyte is at an extreme low or high, it can be fatal (Senior, 1989). Therefore, the nontoxic effect of the aqueous methanol leaf extract of *senna occidentalis* at almost all the doses investigated on the renal function indices may suggest that the normal functioning of the nephrons at the tubular andglomerular levels were not affected. However, a significant increase in creatinine (100 mg/kg dose) may not be due to the excessive amount of water intake. A significant increase in urea and creatinine (100, 200, and 300 mg/kg doses) could be as a result of water overload. Moreover, plasma urea concentration is less reliable than creatinine as an index of glomerulus filtration rate. Increase or decrease in the levels of electrolytes within serum may be a consequence of the hypo or hyper functioning of the concerned organ or tissue. Sodium, potassium, and chloride are examples of clinical electrolytes used in assessing kidney function (Gowla *et al.,* 2010). Sodium is the major cation of the extracellular fluid where it regulates acid-base equilibrium and protects thebody against excessive fluid loss.

Hypernatraemia though rare, may occur in dehydration and steroid hormone administraton.Hyponatraemia, on the other hand, is more common and may be due to chronic sodium losing nephropathy. Loss of gastrointestinal secretion through diarrhea or vomiting, loss from skin as result of burns, loss from kidneys through the use of diuretics and metabolic loss through starvation or diabetic ketoacidosis (Tilkam *et al.,* 1979). From the table 4.5. The significant reduction in chloride may be due to metabolic loss through starvation, but not due to

the extract. Potassium is the major intracellular cation with similar roles to those of sodium. Excessive renal loss of potassium is associated with diuresis, renal loss as a result of potassium losing nephropathy or as a result of renal tubular acidosis. Other causes of hypokalaemia `include excessive loss without adequate replacement as in chronic diarrhoea, malabsorption syndrome, perspiration and chronic fever, chronic stress, poor dietary habit, Cushing's syndrome, hyperaldosteronism, liverdisease with ascites, use of some drugs such as indomethacin, phenylbutazone and steroid hormone (Eccles, 1993). Hyperkalaemia is usually encountered frequently in renal failure, improper use of K+ sparing diuretics, hypoaldosteronism, insulin deficiency associated hyperglycaemia, Addison's disease, and massive tissue destruction. Potassium is used as the mostconvincing electrolyte marker of renal failure. The combination of decreased filtration and decreased secretion of potassium in distal tubule during renal failure cause increased plasmapotassium (Gowda *et al.,* 2010). Hyperkalaemia is the most significant and life-threatening complication of renal failure (James and Mitchel, 2006). From the present study, there was no significant difference in potassium, but chloride in all the tested dose.

CHAPTER FIVE
SUMMARY, CONCLUSION AND RECOMMENDATION

5.1 Summary

The leaves of *Senna occidentalis*leaf were air dried, grounded and soaked in aqueous methanol to get an aqueous methanol crude extract, which is then further tested against *Plasmodium falciparum*,andalso its impact on kidney and liver function was assessed. The crude aqueous methanol extract was then partitioned in to Ethyl acetate, chloroform and N-hexane fractions. The chloroform partitioned fraction being the most active was tested against *Plasmodium falciparum* which was further subjected to characterization using GC-MS. The results shows that chloroform partitioned extract have the highest elimination with a percentage parasitemia clearance of 94.64 %. The chloroform fraction was then characterized using GC-MS yielding polyunsaturated, esters and Lup-20(29)-en-3-ol, acetate, Urs-12-en-3-ol, acetate (3 beta) and 12-Oleanen-3-yl acetate, (3.alpha**).** Administration of the aqueous methanol leaf extract of *Senna occidentalis* to rat does not show little on effect on kidney and liver indices.

5.2 Conclusion

The result of this study suggests the potentials of*Senna occidentalis*for use in the treatment of malaria. The results shows that chloroform partitioned have the highest anti-plasmodial activity. The chloroform extracts was found to contain Lup-20(29)-en-3-ol, acetate, Urs-12-en-3-ol,acetate(3 beta) and 12-Oleanen-3-yl acetate, (3.alpha) which are reported to posses anti-malarial activity.Therefore providing scientific basis for using *Senna occidentalis* as an antimarial plant.it was also established that the *senna occidentalis* has little effect on kidney and liver this indicates that the leaf are safe for human consumption and could therefore be used for antiplasmodial theraphy

5.3 Recommendation

The presence of various bioactive compounds might be associated with the antimalarial effect of Aqueous Methanol leaf extract of *senna occidentalis*leaves. It is recommended for further research studies to isolate, purify, and to characterize a single compound for the development of an antimalarial drug.

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APPENDIX I

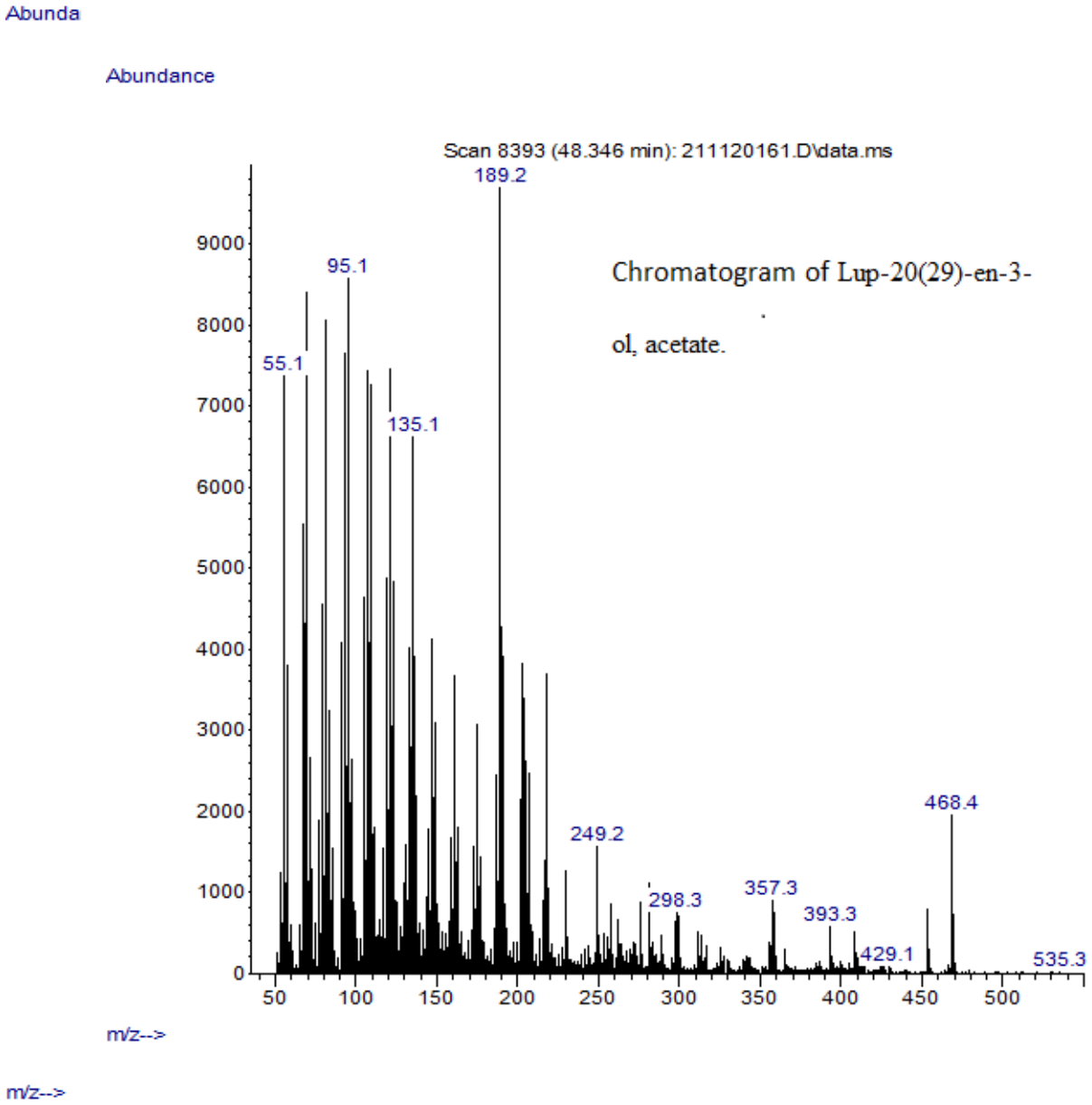

Table 2 shows the possible functional groups of aqueous-methanolic crude root extract of *Senna occidentalis*, twenty two (16) peaks were presented which were overlap to ten (8) peaks that has close similarity indices with the following compounds of standard libraries.

APPENDIX II

List of equipment used for the research work

EQUIPMENT	MODEL	MANUFACTURER
Centrifuge	800D	Gulfex medical and scientific England
Spectrophotometer	Spectrum Lab &52s	News life medical instrument England
Water bath	OLS 200	Grant instruments (Cambrdige) LTD.
Weighing balance	Scout Pro Spu402	Ohaus Corporation, Pine Brook, NJ USA
deep freezer		Thermocool Nigeria LTD
Mortar and pestle		
Animal cage		
Stop watch		
Seiver		
Cuvette		
Micropipette		
Test-tubes		
Syringe		
Razor blade		
Sample bottle		
Plane serum bottle		
Hand gloves		
Cotton wool		
Laboratory glasswares		

APPENDIX III

List of chemical with their respective % purity used for the research work were listed below

CHEMICALS	%PURITY	MANUFACTURER
Chloroform	99.0%	BDH
Ethyl acetate	99.0%	BDH
Hexane	99.0%	BDH
Methanol	99.0%	BDH
Ammonia	98.9%	M & B
Anhydrous sodium sulphate	99.6%	BDH
Methyl red	99.5%	M & B
Ethanol	99.7%	BDH
Sodium thiosulphate	99.5%	BDH
Gelatin solution	99.0%	OXOID
Acetone	99.5%	East Anglia chemical Ltd
Anhydrous calcium chloride	90.0%	BDH
Picric acid	99.5%	M & B
Lead acetate	99.0%	BDH

APPENDIX IV

List of commercially prepared kits used in the research

Aspartate aminotransferase (AST) Kit- Randox laboratories Ltd, United Kingdom

Alanine aminotransferase (AST) Kit- Randox laboratories Ltd, United Kingdom

Alkaline Phosphatase (ALP) Kit- Randox laboratories Ltd, United Kingdom

Albumin (ALB) Kit- Randox laboratories Ltd, United Kingdom

Bilirubin (BIL) Kit- Randox laboratories Ltd, United Kingdom

Urea (UR) Kit- Randox laboratories Ltd, United Kingdom

Creatinine (CREA) Kit- Randox laboratories Ltd, United Kingdom

Potassium kit-TECO diagnostics, Netherlands.

Chloride kit-TECO diagnostics, Netherlands.

Bicarbonate Kit-TECO diagnostics Netherlands.

Sodium Kit-TECO diagnostics Netherlands.